THE CIRCULATORY AND NERVOUS SYSTEMS OF THE GIANT SCALLOP (PECTEN TENUICOSTATUS, MIGHELS), WITH REMARKS ON THE POSSIBLE ANCESTRY OF THE LAMELLIBRANCHIATA, AND ON A METHOD FOR MAKING SERIES OF ANATOMICAL DRAWINGS.

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In considering any system of organs it is essential that we should bear in mind the modifications of the possessor of the organs, that adapt it to its particular life.

Pecten is one of the ablest swimmers among lamellibranchs. The whole structure of the animal is modified for this purpose. The valves have become rounded in outline, flattened, and comparatively light. The anterior adductor muscle has been lost, and the posterior adductor muscle, which is very powerful, is situated near the middle of the body. The cartilage has become well developed, so the shell may be opened quickly when the muscle relaxes, and the hinge line is straight, so there may be no unnecessary strains in opening and in closing the shell. Each gill is attached by one lamella only, so water in the temporary cloacal chamber may be thrown out without injuring the gills, and the gills and margins of the mantle are provided with muscles to withdraw them from the margins of the shell when the shell is closed. Furthermore the margins of the mantle are provided with infolded ridges and with circular muscles so it is possible to direct the current of water which issues from the shell in the required direction.

To fit the animal to a life of such activity, and to enable it to live in the comparatively exposed positions that it inhabits, an abundance of sense organs, tactile and probably visual, have been developed. These are placed in the most exposed positions, where they may give warning to their possessor, and are

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[1] Free use has been made of both descriptions and figures published as No. 6 of the University of Maine Studies, under the title of "The Habits, Anatomy, and Embryology of the Giant Scallop (*Pecten tenuicostatus*, Mighels)."

accordingly borne along the margins of the lobes of the mantle.

It is not entirely certain what relationship *Pecten* bears to the usual form of lamellibranch as regards positions of parts. In lamellibranchs that are supplied with two practically equal adductor muscles, a line connecting the two adductors runs nearly lengthwise of the animal. In such a case the hinge line is more or less dorsal, one end is anterior, and the other posterior. When one of the muscles disappears, as is the case with *Pecten*, one of the landmarks disappears and it becomes more difficult to locate the direction of parts. Inasmuch as the hinge line is usually dorsal, it is very natural to look at the hinge line of this form as dorsal, and for matters of description it is convenient to so consider it. If, however, the position that the anterior adductor would have occupied, had it been retained, be considered, the position of the mouth, foot and heart indicate that it would have to be placed much nearer the hinge line than the present position of the posterior adductor muscle, the muscle that is retained. If this is the case, it becomes evident that the loss of the anterior adductor muscle has been accompanied by a general reduction of the anterior part of the body, so a large part of the body of *Pecten* is to be considered morphologically posterior. This supposition seems to be borne out by the nervous system, and the vascular system of the mantle, as well as by the extent and position of organs. In most forms the margin of each lobe of the mantle is supplied with a posterior and an anterior pallial nerve of approximately equal size. These nerves supply the muscles and sense organs of the margins, and, in many forms at least, unite with each other so they form a continuous connective between the cerebral and the visceral ganglia. In *Pecten*, not only is this the case, but the nerve in the margin of the mantle is joined at intervals for nearly its whole length by nerves from the visceral ganglion (Fig. 6). On the other hand, it is joined only in the region of the anterior ear by nerves from the cerebral ganglion. The visceral ganglia are the important ganglia of the animal, and both the cerebral and pedal ganglia are greatly reduced.

The blood is supplied to the mantle very largely by the posterior pallial arteries (Fig. 5). The anterior pallial arteries are comparatively small, and while they are connected with the posterior pallial arteries, the size and character of the vessels indicates that the junction is probably very near the anterior ear.

Considering everything, it seems likely that the longitudinal axis of the body could be morphologically represented by a line drawn from near the hinge extremity of the anterior ear to the middle of the adductor muscle, and that a very small portion of the scallop is anterior.

Circulatory System.

The animal is large enough to allow one to successfully inject the chief vessels with starch or gelatin injecting mass, and then by dissection and microscopic preparations to trace the distribution of the vessels of the different organs and to determine quite definitely the course taken by the blood in its circulation.

The heart is a typical, symmetrical lamellibranch heart, with two auricles and one ventricle (Figs. 1 and 3), the latter perforated by the intestine which enters it near one end and leaves it near the other end (Fig. 2). Dorsally the ventricle is prolonged somewhat, posterior to the intestine, where the morphologically anterior aorta is given off, and ventrally to a less extent it is prolonged anterior to the intestine, where the much smaller morphologically posterior aorta is given off. The walls of the ventricle are of about even thickness throughout their extent, and are quite smooth outside and inside. The auricles join the ventricle on each side near its middle, are somewhat triangular in shape, with the most acute angle receiving blood from the gills and mantle at a point dorsal to the adductor muscle and directly ventral to, but some distance from, the cartilage. The opening of each auricle into the ventricle is near the middle of the side of the auricle that lies next to the ventricle and farthest away from the opening where the auricle receives its blood. The muscles around the openings of the auricles into the ventricle, and to a less extent around the openings through which the auricles receive blood, are well developed and must act as sphincters that tend to keep the blood from being regurgitated The walls of the auricles, unlike those of the ventricles, are roughened by pits that open into the cavities of the auricles.

Both auricles and ventricle are composed of interlacing muscle fibers, and are capable of great extension. In preserved specimens, the heart is usually contracted and is not very conspicuous. In such contracted hearts the cavities of auricles and ventricle are practically obliterated.

The heart lies in a somewhat triangular, spacious pericardial cavity that is dorsal to the posterior half of the adductor muscle, and ventral to the posterior portion of the liver. Posteriorly it is covered only by a somewhat thick, muscular membrane which separates it from the mantle chamber.

As already mentioned, two blood vessels leave the ventricle (Figs. 1 and 3), one from each end. Although they are not so placed in reference to the ways the terms are generally used in describing *Pecten*, the two ends correspond to the anterior and posterior ends of the ventricle in most forms of lamellibranchs. The posterior aorta is much the smaller of the two, leaves the heart ventral to the intestine (actually anterior to it) and divides immediately after leaving the heart, into two vessels, one of which, the smaller, follows along the intestine, supplying it and surrounding portions with blood. The other vessel turns almost at right angles upon leaving the aorta and enters the adductor muscle, where it divides into a system of vessels that supply the muscle with blood.

The anterior aorta is much larger than the posterior aorta, and supplies all of the remainder of the body. It leaves the ventricle dorsal to (actually posterior to) the intestine and very soon gives rise to a vessel which passes into and supplies the wall that separates the pericardial cavity from the mantle chamber. From the pericardium the anterior aorta follows along the postero-dorsal border of the liver to the base of the ear. Here it gives rise to the branch (Fig 3, *ppa*) which passes posteriorly to the extreme upper margin of the mantle that lines the ear, giving off along its course a number of branches, which supply this portion of the mantle. Here it divides into two vessels, a right and a left, each of which bends abruptly ventrally (Fig. 5, *ppa*) and follows along the margin of the respective mantle lobe about opposite the line of attachment of the infolded ridge of the mantle, alongside but external to the pallial nerve.

Very fine branches are given off from these vessels all along their courses, which further divide to form systems of capillary spaces that are finest and most numerous near the margins. Some of these capillary spaces are large enough to be injected with starch mass, and I have a preparation of the mantle lobe from which only the infolded ridge has been removed, that was dehydrated, cleared and mounted in balsam, in which the whole system of vessels can be traced. A gelatin mass not only fills the spaces mentioned, but passes out between the cells so that in sections it may be seen to be diffused throughout the tissue. This seems to hold good for all other parts of the body with the exception of the gills, in which organs the mass is more completely, but not entirely, confined to the blood spaces. The indication therefore is, that the blood spaces are not confined vessels, and that the blood functions as both blood and lymph. The posterior pallial vessel may be traced far anteriorly, gradually diminishing in size along its course. Here it finally joins the anterior pallial vessel. The anterior pallial artery (Fig. 3, *apa*) leaves the anterior aorta very near the cartilage and runs directly to the anterior border of the hinge region of the mantle, giving off vessels to this portion of the mantle on the way. Here it branches into right and left vessels, each of which bends abruptly ventrally (Fig. 5, *apa*) and pursues a course along the anterior border of the mantle similar to that taken by the posterior pallial artery at the other extremity of the animal.

Along the anterior border of the mantle, near the dorsal line, the vessel is rather small and slightly broken in its course. It may be possible that this represents the border line between the posterior and the anterior pallial arteries. There are other reasons for believing that a large share of the animal is morphologically equivalent to the posterior portions of other forms, and that the anterior portion is greatly reduced. This has received attention in another place.

Several vessels leave the anterior aorta to supply the liver and stomach. Most prominent among these is a vessel which leaves the aorta between the points of origin of the anterior and posterior pallial arteries. This bends out toward the left side of the liver, where, in injected specimens, it is very conspicuous, passes ventrally and sends branches to the major part of the liver and to the stomach.

A short distance in front of the cartilage the anterior aorta bends ventrally, passes through the liver and gives off a few small branches to it, sends a vessel to the palps in passing, and passes on to supply the foot and the visceral mass. The vessel that supplies the foot (Fig. 3, *fa*) leaves the aorta a short distance ventral to the mouth, passes along the body wall until the foot is reached, and extends into the foot along its dorsal border. Just before entering the foot this, the pedal artery, gives rise to a small vessel that passes posteriorly along the single retractor muscle of the foot, supplying it with blood. From the point of origin of the pedal artery the aorta extends into the visceral mass, following along the enlarged portion of the intestine that leads away from the stomach, and supplying this and other portions of the intestine and the reproductive organs with small and with large branches. The enlarged portion of the intestine that comes from the stomach is especially well supplied (compare Figs. 2 and 3), there being numerous small branches that are given off directly from the aorta, and large branches that follow along on the different sides of this portion of the intestine and likewise supply it with branches. A short distance ventral to the foot a large branch leaves the aorta and passes postero-ventrally to divide again and form small branches that supply the remaining loops of the intestine and the postero-ventral portions of the reproductive organs.

This completes what might be called the systemic arterial system. Beginning with the heart the system ends in the capillary spaces of the various organs. This system is most easily injected through the vessel in the suspensory membrane of the gills that is farthest from the adductor muscle (Fig. 1, *bv*), with a hypodermic syringe, injecting toward the heart. If a starch mass that will not pass through the capillary spaces is used, all of the vessels thus far described will be injected, as will also the veins that return blood from the gills, as this vessel is the one that returns blood from the gills to the heart. If a gelatin mass is used all of the systems may be injected, but as the injecting mass may pass out of the spaces, between the cells of the various organs, such injection does not aid in tracing the course of blood flow.

The systemic veins (Fig. 4) that collect the blood that is supplied by the systemic arteries, from the various organs of the body, may be injected from several different vessels. They may be injected by pushing the needle beneath the membrane that covers the posterior surface of the adductor muscle. A large blood space occupies this position, into which the needle is inserted and the mass injected fills the systemic veins. Another point from which these veins may be injected is from one of the superficial vessels of the visceral mass. These vessels are very conspicuous, and may be very easily picked up with the needle. Still another vessel is the vein that returns blood from the liver, which may be seen on the left side of the animal anterior to, but near the large artery that supplies the liver. Injecting any one of these vessels will to a greater or less extent inject the others, but there does not seem to be an entirely free communication between them. They all carry blood to the kidneys, and seem to empty into a common sinus on either side, that lies alongside the kidneys in the walls of the visceral mass. The sinuses of the two sides are connected beneath the adductor muscle, but it frequently happens that a complete injection of the system is not obtained from an injection from any one of the veins mentioned. Just where the obstruction lies in such cases has not been determined. It has been noticed that obstructions are more likely to be encountered in injecting from the veins of the visceral mass than in injecting from any of the others.

Inasmuch as blood spaces are cut in removing the muscle from the shell, it has been found desirable in injecting this system of vessels to wedge the valves open and to inject from the posterior surface of the adductor muscle. In injecting after the animal is removed, a considerable quantity of the injecting mass is sure to escape at the ends of the muscle.

The position of the veins may be seen in Fig. 4. A large vein comes from the liver, another from the foot, and the veins in the muscle unite to form a more or less definite sinus along the dorsal border of the muscle, and two smaller ones on the antero-ventral side of the muscle. These sinuses unite near the anterior ends of the kidneys. A series of vessels from the visceral mass unite along the borders of the kidneys and finally connect with these sinuses. Most of the blood from all of these organs is distributed to the kidneys through systems of capillary spaces. The branching of these vessels is not conspicuous on the surface of the kidneys, but is better seen by cutting the kidneys open. That not all of the blood necessarily traverses the capillary spaces of the kidneys is indicated by the fact that injections of the systemic veins frequently fill the veins that carry the blood away from the kidneys as well as those leading to it. This is much more frequently the case when injecting from the posterior surface of the adductor muscle than when injecting from other places, and seems to be dependent upon a direct connection between the vessel in question and the sinuses on the antero-ventral surface of the adductor muscle near the dorsal ends of the kidneys.

Of the blood that leaves the heart, only that which goes to the mantle remains to be accounted for. This is collected and returned directly to the heart (Fig. 5, *pv*.)

All of the blood that leaves the kidneys is conducted to the gills. The blood from each kidney is collected into a sinus that runs along the border of the kidney that is applied to the adductor muscle. This sinus, which also seems to receive blood from the sinuses on the anterior and ventral surfaces of the adductor muscle, bends abruptly ventrally over the anterior end of the kidney and is continued on the lower border of the suspensory membrane of the gill (Fig. 1, *ba*) to the posterior end of the gill, supplying the gill with branches throughout its length.

Blood vessels leave the vessel that carries blood from the kidney, opposite each of the inter-lamellar junctions of each of the gills supported by the suspensory membrane. Each of these branches is continued along the free border of the membrane that forms the inter-lamellar junction (Fig. 7, *ba'*) until it reaches the free edge of the lamella, the edge that is not attached to the suspensory membrane. That is, if the branch supplies an outer gill, it leaves the suspensory membrane along the free border of an inter-lamellar junction and crosses over to the free border of the outer lamella of this gill. Here the vessel is continued down the enlarged, modified filament that is concerned in the formation of the inter-lamellar junction (Fig. 7, *ba''*) giving out side branches through each of the inter-filamentar junctions (as long as these are composed of tissue that can carry blood vessels)[1] and so supplies the various filaments of the lamella. The blood thus distributed finds its way around the margin of the gill through small blood spaces and is continued up the other lamella of the gill, the blood of the small filaments being gradually collected through the vessels of the inter-filamentar junctions into the vessels of the large filaments (Fig. 7, *bv′*), and by these poured into a vessel that lies just beneath the vessel that supplies the gill and runs parallel with it (Fig. 1, *bv*). This vessel receives all of the blood from both of the gills of the side, and carries it directly to the corresponding auricle of the heart. Just before the vessel empties into the heart it receives a rather large vessel from the corresponding lobe of the mantle, which returns the blood that was sent to the mantle back of the heart.

To sum up the course of the circulation of the blood briefly, it will be seen that of the blood that leaves the heart only that which is sent to the mantle is returned to the heart after traversing a single set of capillary spaces; that a small portion of the blood sent to the adductor muscle (that which is collected by the sinuses on the antero-ventral portion of the muscle) may be returned after traversing two sets of capillaries — those of the adductor muscle and those of the gills; and that the greater portion is returned only after traversing three sets of capillaries — those of the general system, those of the kidneys, and those of the gills.

The reasons for this arrangement of the circulatory system are at least in part not hard to find. The blood which passes to the mantle loses some of its nourishing materials, but as the mantle lobes are thin and are bathed over such a large portion of their surfaces by a current of water, in which there is an abundance of dissolved oxygen, respiration, no doubt, takes place direct, and the blood has no need to pass through the gills to get a supply. Again the work of the mantle is not of such an active nature as to load the blood with nitrogenous wastes. It seems likely that the amount of nitrogenous waste in the blood that has traversed the mantle is so small that it

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[1] The inter-filamentar junctions near the free margins of the gills are composed of cilia only.

would diminish the proportion of nitrogenous waste in the blood, if this blood were added to the blood that passes through the kidneys.

The blood that goes to the general system must in its progress lose a considerable portion of its oxygen, and in all portions except around the alimentary canal (where there is, of course, a decided gain) also food materials, and gain from the tissues a considerable amount of nitrogenous and carbonaceous wastes. It is then essential that such blood should go to the excretory and respiratory organs to get rid of these waste products and to gain oxygen. Inasmuch as the heart provides for but a single circulation it is necessary that the capillaries of these organs be traversed before the blood is returned to the heart. Why it is arranged so part of the blood may dodge the kidneys and be carried directly to the gills is not nearly so evident. Possibly the periodically great activity of the adductor muscle causes the blood to move through it so rapidly that the small kidneys cannot take care of it and properly perform their function, and the other channel is provided to carry the surplus away to the comparatively extensive gills where the increased flow can be taken care of with greater ease. It is, of course, essential that the amount of oxygen in the blood at such times shall not be reduced. It is at any rate evident that there is a possibility that part of the blood that is returned from the muscle, liver, etc., may not pass through the kidneys, for when starch injecting mass is injected through a vessel that carries blood from one of the kidneys to the gills, not only are the kidney and the gills injected. but part of the mass usually finds its way into the adductor muscle, liver, and other organs of the body.

The rate of the heart beat is slow, and as in other lamellibranchs is, no doubt, dependent upon the temperature of the animal as well as on other factors. The auricles and ventricle become very greatly distended during diastole, and contract so that their cavities are almost entirely obliterated in systole.

NERVOUS SYSTEM.

The three pairs of ganglia that are usually found in lamellibranchs are present in this form, but they differ greatly in size and they are not all placed in the usual positions.

The cerebral ganglia (Fig. 6, *cg*) are placed some distance ventral to the mouth, just beneath the outer covering of the body. They, like the other ganglia, are yellowish in color, and may frequently be faintly seen through the covering of the body. Each cerebral ganglion is somewhat elliptical in outline with the long axis directed dorso-ventrally and has a rather distinct swelling on the ventral (actually anterior) and outer side (the side away from the median plane of the body) (Fig. 9, *cg*). The anterior end of each cerebral ganglion presents a forked appearance, due to the origin of two large nerve cords. The inner and ventral one of these two cords (Figs. 8 and 9, *cc*) is the commissure that joins the two cerebral ganglia. As the ganglia lie some distance ventral to the œsophagus, this commissure forms a long loop that passes dorsally around the œsophagus just posterior to the mouth. The outer and posterior of the two large cords that leave the anterior end of each ganglion is the anterior pallial nerve (Figs. 6, 8 and 9, *apn*). This runs parallel with the commissure as far as the œsophagus and is then continued along the side of the liver, and in the mantle, to the margin of the mantle in the region of the anterior ear of the shell, where it joins by several branches the circumpallial nerve (*cpn*) that follows along the margin of the mantle near the bases of the tentacles and eyes. The circumpallial nerve will receive attention later.

Between the points of origin of the cerebral commissure and the pallial nerve, a small nerve (Figs. 8 and 9, *pn*) leaves the ganglion to be continued dorsally, and to supply the labial palp.

From the inner, ventral surface of each cerebral ganglion, a little in front of the middle, the cerebro-pedal connective leaves to join the pedal ganglion of the same side. The cerebro-pedal connective is smaller near the cerebral than the pedal ganglion (Fig. 9, *cpc*) and bears a ganglionic swelling on its outer side very near the pedal ganglion.

In the acute angle formed by the surface ot the cerebral ganglion with the cerebro-pedal connective, a small nerve (*otn*), the otocystic nerve, leaves the ganglion to be continued around the dorsal surface of the cerebro-pedal connective to the otocyst of the same side.

Posteriorly the cerebral ganglia taper rather gradually into the cerebro-visceral connectives, which run along the sides of the visceral mass very near the adductor muscle, until the visceral ganglia are reached.

The pedal ganglia lie very near each other (Fig. 9, *pg*), so the commissure that connects them is short and broad and presents ordinary ganglionic structure. They are separated from the cerebral ganglia only by a short interval, and lie anterior and slightly ventral to them, some distance dorsal to the base of the foot. They lie so near the surface that their color may frequently be distinguished through the body wall beneath the mouth. Two large nerves (*fn*) leave each pedal ganglion to be continued into the foot, where they supply the muscles of the foot and probably the byssal gland. The swellings on the cerebro-pedal connectives near the pedal ganglia have already been described. The otocystic nerves, which usually leave the cerebro-pedal connectives near the pedal ganglia, in this form originate directly from the cerebral ganglia near the point where the connectives leave the ganglia.

The visceral ganglia (Figs. 6, 8 and 10, *vg*) are by far the largest and most complicated of the ganglia, and from them nerves are sent to most parts of the body. They are situated on the antero-ventral surface of the adductor muscle, nearly opposite the external openings of the kidneys. They are imbedded in a mass of connective tissue and are fused to each other, so the commissure that connects them is nearly as broad as the ganglia themselves and shows ganglionic structure. The chief indication of the presence of a pair of ganglia is the arrangement of the nerves that leave them, and of the cerebro-visceral connectives that join them. The ganglia are divided into very definite regions, each of which is connected with definite bundles of nerve fibers and, no doubt, has a particular function to perform. I have not had time to make a detailed study of the structure and nerve tracts of the ganglia, but I am satisfied that there is much more complexity than is ordinarily attributed to the ganglia of lamellibranchs. The dorsal surfaces of the ganglia are quite smooth, but when seen from the ventral surface (Fig. 10) the regions that are indicated in the figure are always visible. On each cerebro-visceral connective, just before it joins the ganglion proper, there is a ganglionic swelling (*x*) that supplies one of two roots of a nerve (Figs. 6, 8, and 10, *bn*) that leaves in an antero-dorsal direction along the border of the excretory organ, to bend ventrally and posteriorly in the suspensory membrane of the gills, and supply the gills of the corresponding side. Between the points where the cerebro-visceral connectives join the visceral ganglia on the ventral side, there are four rather distinct swellings, with three less distinct swellings posterior to them. Extending laterally from the outer side of each ganglion is a somewhat flattened ridge (Fig. 10, *y*) from which all of the pallial nerves from this ganglion originate. These nerves (Figs. 6 and 8, *ppn*) pass laterally, posteriorly and anteriorly along the surface of the adductor muscle, to meet the mantle lobes and to be continued to the margins, where they unite with the circumpallial nerves. It will be noticed that they unite with the circumpallial nerve at intervals throughout the greater length of this nerve. As the pallial nerves that leave the visceral ganglia in most forms pass directly to the posterior portion of the mantle, the distribution in this form may be looked upon as evidence that all of this portion of the mantle belongs morphologically to the posterior portion of the animal.

Other nerves leave the dorsal surface of the visceral ganglia near their posterior ends, and enter the adductor muscle directly. The nerves that supply the posterior division of the muscle are continued along the ventral surface of the anterior portion of the adductor muscle until this posterior portion is reached. Small nerves also leave the ventral side of the ganglia and penetrate the visceral mass.

All of the ganglia are well supplied with nerve cells, there being very many large polar cells present, but the number of the cells is far greater and their arrangement more complicated in the visceral than in any of the other ganglia.

Nerve cells are also to be found in the circumpallial nerves and in the branchial nerves. So abundant are the nerve cells in the circumpallial nerves that they assume the structure of ganglia. The nerves by which they are connected with the visceral and cerebral ganglia contain no ganglionic cells. From the structural standpoint we would accordingly be justified in considering the circumpallial nerves as separate ganglia, and the nerves connecting them with the visceral and cerebral ganglia as connectives.

The circumpallial nerves of the two lobes of the mantle are connected with each other anteriorly and posteriorly near the hinge line (Fig. 8, *cpn*). They are not of constant diameter, but suddenly increase or diminish in size so that they have a rather irregular appearance. They lie just inside, that is, toward the median plane of the body, of the large pallial arteries that supply the mantle margin, about opposite the line of attachment of the infolded ridge. From them nerves are sent to the eyes and tentacles, to the infolded ridge and to the pallial muscles. Very likely the pallial muscles are partially supplied from the pallial nerves that come from the visceral ganglia, but of this I am not sure.

It seems probable that the ganglionic structure of these nerves has been developed to meet the needs of the very complex margins of the mantle. The development of ganglia in the immediate region of the sense organs is an indication of the ease with which such centers may be established when need arises.

The branchial nerves are supplied with ganglionic cells throughout their length. These are present not only along the borders of the gills, but from the points where the nerves originate to their extremities. The almost constant activity of the gills no doubt renders such an arrangement desirable. No other nerves or connectives in the body seem to be abundantly supplied with ganglion cells.

The whole nervous system is modified to meet the special needs of the animal. The cerebral and pedal ganglia are small, corresponding with the slight development of the anterior parts of the body and of the foot. The visceral ganglia are highly developed, corresponding to the excessive development of the parts that are supplied by these ganglia. Accessory centers have also been developed in the margins of the mantle and in the gills.

It seems that many students of Mollusca hold that the lamellibranch ganglia have been derived from a gastropod-like type, a type that possesses at least one pair of ganglia, the pleural, that are not commonly found in lamellibranchs. This view seems to be based largely upon the acceptance of a hypothetical type for a primitive mollusk that seems to me to be a much better ancestor for the gastropods than for the other classes of the Mollusca.

PHYLOGENY.

The hypothetical primitive mollusk that has persistently been offered for our consideration, and has found its way into a number of text-books, among which is Lang's "Text-Book of Comparative Anatomy," has the dorsal portion of the body covered by a conical shell, the foot flattened and adapted for creeping, a head fold that may be protruded from beneath the shell, a pair of plumose gills, and a nervous system with at least four pairs of definite ganglia, cerebral, pleural, pedal and visceral. *Distinctly gastropod throughout.*

If the development of animals is to be considered of any importance in pointing their possible lines of descent, and as long as embryo chicks have gill arches our belief has good foundation, it would seem that in those mollusks whose eggs are not loaded with yolk, whose embryos are not modified for protection in brood pouches, and do not have long larval histories that call for special modifications to enable them to cope with enemies and to get food, the embryos might be suggestive.

The presence of unlimited food and protection always tend to destroy characters. Thus we find that parasitic forms may have entirely lost organs that must have been well developed before the animals took to parasitic lives. The presence of a quantity of yolk furthermore frequently must have mechanical effects on the developing embryo that cause direct modification. Again those embryos that pass through long larval histories exposed to the competition of forms that would eat their food and other forms that would eat them, must necessarily be exposed to the same evolutionary factors, whatever they may be, that adult animals are exposed to and we would accordingly expect adaptive modifications in them.

There are many lamellibranchs, and not a few gastropods, that do not seem to be seriously modified by any of these factors and when their embryos are examined every one must be struck with their close resemblances. These embryos would seem to point to a free swimming ancestral form that obtained its food by means of surface cilia.

The first living forms that made their appearance on the earth must have used non-living substances for food. What the nature of these substances were, whether they were of a comparatively simple nature, like those that are used by our green plants to-day, or whether they were of an entirely different nature, we have no means of knowing, but it is evident that their food was not alive.

Then came the discovery by some form that the protoplasm of other forms could be used for food. This must have been the first great factor that led to the competition of forms and called for the improvement of bodily machinery among living things, to aid in the struggle thus begun, the struggle to get food and to escape from being used as food. As Professor Brooks has indicated,[1] this would naturally lead to the discovery and colonization of the bottom of the ocean because of the greater advantages it offered both for capturing food and in affording means of protection. This introduces the further element into the competition, of some positions being far more favorable than others, and as the struggle for position increased, a struggle that has never ceased, the competition, especially between close relatives, must have become very severe.

These factors, with the struggle dependent upon them, must have caused changes in structure (in the improved machinery that aids forms in getting food and in keeping from being used as food) to change very rapidly and it seems very plausible that in a comparatively short time in those days when forms were of simple structure and this keen competition was begun, the foundations of the great types of animal structure were laid.

We know that among our earliest fossils are to be found both lamellibranchs and gastropods, and it is back in the earlier time that we must look for the changes that have resulted in the formation of these classes.

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[1] Brooks, "The Origin of the Oldest Fossils and the Discovery of the Bottom of the Ocean," Smithsonian Report for 1894 (also Salpa).

We may possibly conceive that the ancestor of the Mollusca was among the early ones to recognize the advantages of the ocean bottom, and that its race soon developed a protective shell, if this had not started to form before it became a dweller on the bottom. The shell would offer protection, but would, because of weight, interfere with rapid movement. As enemies became able to get beneath its armor the shell became thickened and was made to cover the animal more completely, but the added weight interfered still more with rapid movement.

At this time we need not suppose that the animal had more than the very simplest nervous system, hardly more than that needed by a trocophore larva, for it would probably be dependent upon simple bands of cilia, or at the most a movable mouth portion, for getting its food. There is no reason for supposing that this animal had yet developed gills, or if gills were present they would hardly be more than simple folds of the mantle.

As competition became more severe, animals of this kind were in need of better protection, and it is possible to conceive that there might have been evolved two types, one that inclosed itself in a bivalve shell, crawled into the mud, and obtained its food by capturing the forms brought to it in a current of water of its own creation, the other, more like the *hypothetical primitive mollusk that has been described*, which retained a single shell and got its food by creeping over the bottom and picking it up directly. The first form would still have a simple head apparatus and would need new nervous centers only to provide for the mechanism necessary to crawl into the mud and the mechanisms necessary to create the current of water and capture the living forms from it. The second form would have a more complicated head apparatus and would need nervous centers to supply it and to supply the organ by means of which it was enabled to creep. In these differences in life, and in the consequent differences in structure, it seems reasonable to look for the differences in their nervous systems. If this conception is anything like true, from very early times there was no similarity in the method these two groups used in getting food. One has finally developed a remarkably satisfactory method of straining out living particles that serve it as food, from a current of water of its own formation, and is thus able to leave little of its surface exposed to the attacks of enemies. The other has developed one of the most complicated of machines in connection with its mouth to aid it in getting food.

As the head apparatus of the one type has increased in complexity, there has been greater need of ganglia to supply it, but in the whole line of development of the other type there has been no complicated head apparatus. About all of the actual evidence that we have of the presence of pleural ganglia in lamellibranchs is that given by Pelseneer,[1] who finds in *Nucula* and some other forms, that each anterior ganglionic mass is so shaped that it is possible to consider it as two ganglionic masses, and further that the connective that runs from this mass to the pedal ganglion is connected with this mass by two roots. The interpretation that he has put on this is that the two apparent divisions of the ganglion represent respectively the cerebral and pleural ganglion, and that the roots of the connective represent the cerebro-pedal and pleuro-pedal connectives that have become fused before reaching the pedal ganglion. My own view, discussed in another paper[2] is that the apparent division into two ganglionic masses is superficial, and due to the swellings accompanying the origins of nerves, and that one of the cerebral ends of the connective may be the central end of the otocystic nerve which is fused for the greater part of its length with the connective, but, unlike most forms, is free near the ganglion. This view seemed to me most reasonable as Stempell[3] has found that in *Soleyma togata*, a supposed near relative of *Nucula*, the otocystic nerve arises directly from the cerebral ganglion and is separate from the connective throughout its length. So far as I know, the instance given by Stempell is the only one that has heretofore been reported where the otocystic nerves originate from the cerebral ganglia, and are free from the cerebro-pedal connectives throughout their length. *Pecten tenuicostatus* has the same arrangement. In this form the position of the ganglia, connectives and otocysts is such that it is a very simple matter for the

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[1] Pelseneer, "Contribution á l'étude des Lamellibranchs," *Arch. de Biol.*, XI., 1891.

[2] Drew, "The Life-History of *Nucula delphinodonta*," *Quart. Jour. of Micro. Sci.*, Vol. 44, Part 3, New Series, 1901.

[3] Stempell, "Zur Anatomie von *Solemya togata*," *Zool. Jahrb.*, Bd. XIII., 1899.

otocystic nerves to make direct connection with the cerebral ganglia, but they do not join the ganglia at their nearest points. Instead they are continued around the connectives to join the ganglia in contact with, and posterior to them.

To me it seems probable that the separation into the two groups that have developed into the classes Lamellibranchiata and Gastropoda took place at an early date in the history of the Mollusca, probably before a complicated head apparatus was developed, and while the nervous system was of a very simple nature. If this was the case, we have no reason to search for pleural ganglia in lamellibranchs, for it is very probable that they never had them. In fact were ganglia ever present in this region in lamellibranchs, it would be more reasonable to view them as new formations for special purposes than as direct descendants from, and accordingly homologous with, the pleural ganglia of gastropods. The gastropod and lamellibranch are so different in structure and habits that we may reasonably expect important differences in their nervous systems. Gastropods and cephalopods possess accessory ganglia that have evidently been developed to perform special functions. That such centers may be comparatively easily developed is indicated by the fact that the circumpallial nerves of the scallop are essentially such centers. Is it not then more likely that pleural ganglia have been developed in the groups that need them than that lamellibranchs, which, so far as we know, have never been more complicated than they are to-day, should have formerly possessed these ganglia and have since quite uniformly lost them?

Anatomical Drawings.

It sometimes happens that in making a series of drawings intended to illustrate different organs of the same animal, considerable labor can be saved by using a combination of photograph and ink. The figures of the present paper illustrate this saving much better than is usually the case. To draw the margin of the mantle, with its large number of sense organs, requires both time and patience, and were it necessary to draw it for each of the figures where it necessarily occurs, one would be tempted to abandon it altogether.

It occurred to me, while engaged in drawing this margin, that possibly it could be photographed on a paper of a quality that would allow pen drawing and thus save redrawing it. After some trials a platinum paper was found that met the requirements but I was surprised to find how much blacker Higgins ink was than the blackest print I could make.

Evidently, however, any mark that would take at all in making a zinc etching would print the same color as the rest when being put through the press, so one of the poorest of these photographs was finished with Higgins ink and sent away to have a zinc etching made from it. The result was perfectly satisfactory. It will be seen that the margins on Figs. 1, 2, 3, 4, 6, 11, and 12 are all alike. The margin of Fig. 1 is the only one that was made with pen and ink. Fig. 11 is a print of a negative made from this margin before the rest of the animal was drawn. Taking a print similar to that shown in Fig. 11, with pen and ink there was drawn into it the organs shown in Fig. 12. Fig. 2, before the alimentary canal was added, was the figure from which the photograph resulting in Fig. 12 was taken. The margin of Fig. 12 is then a photograph of a photograph of an ink drawing. The original of Fig. 12 was then worked on to form Fig. 2 just as the original of Fig. 11 was worked into Fig. 1. Figs. 3, 4 and 6 are all worked onto prints similar to that shown in Fig. 12. In the original paper in which these figures were published a number of others were based on photographs in a similar way. The saving of time in the paper probably amounted to more than one half, and certainly may be of importance to others. I have no doubt that photographs may also be made the basis of brush work, but great care will be necessary in such cases in getting the proper printing value. While the figures accompanying this paper show no evidence that the photograph and the ink had different printing valves, they would have been very unsatisfactory had they been reproduced by some other processes.

Summary.

Circulatory System. — The large size of the animal makes it possible to inject the vascular system successfully. Blood from the mantle is returned immediately to the heart. Most of the blood from other portions is carried to the kidneys, from which it is carried to the gills, and then back to the heart. A portion may dodge the kidneys and go to the gills. Blood seems to act both as blood and lymph. (See pp. 227–234 and Figs. 3, 4, 5 and 7.)

Nervous System. — The cerebral and pedal ganglia are small and somewhat removed from their usual positions. The visceral ganglia are very large and complicated in structure. The circumpallial nerves and the branchial nerves have ganglion cells throughout their length. The otocystic nerves originate directly from the cerebral ganglia. (See pp. 234–239 and Figs. 6, 8, 9 and 10.)

Phylogeny. — Ontogeny and the probable conditions that have resulted in the complication of structure, both seem to indicate that the division of the Mollusca into lamellibranchs and gastropods, took place at an early time, before the ancestors had attained much complexity of structure.

There seems to be no reason for believing that lamellibranchs ever had more complicated head machinery than they have at the present time. If this is true they probably have never had need of more anterior ganglia than they now generally have. (See pp. 239–243.)

Anatomical Drawings. — A combination of photographs and drawings may sometimes save much time and tedious work. (See pp. 243 and 244.)

UNIVERSITY OF MAINE,
ORONO, MAINE,
November 15, 1906.

PLATE XVII.

FIG. 1. Animal as seen from the left side with the left shell valve and mantle lobe removed and with a portion of the pericardial wall cut away. A few of the blood vessels are shown. Two thirds natural size.

FIG. 2. Animal as seen from the left side with the left shell valve and mantle lobe removed, with the alimentary canal shown. Two thirds natural size.

a, auricle; *ba*, branchial artery; *bv*, branchial vein; *c*, cartilage; *e*, excretory organ; *f*, foot; *fe*, free edge of the unattached lamella of the gill; *g*, gill; *i*, intestine; *lp*, labial palp; *m*, mantle; *pa*, posterior adductor muscle; *s*, stomach; *v*, ventricle; *vm*, visceral mass.

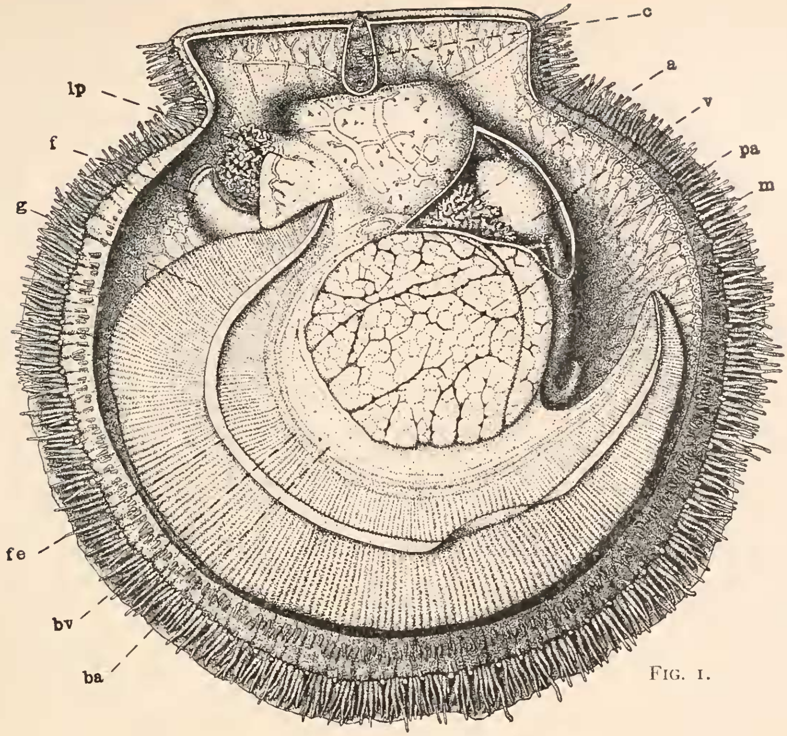

FIG. 1.

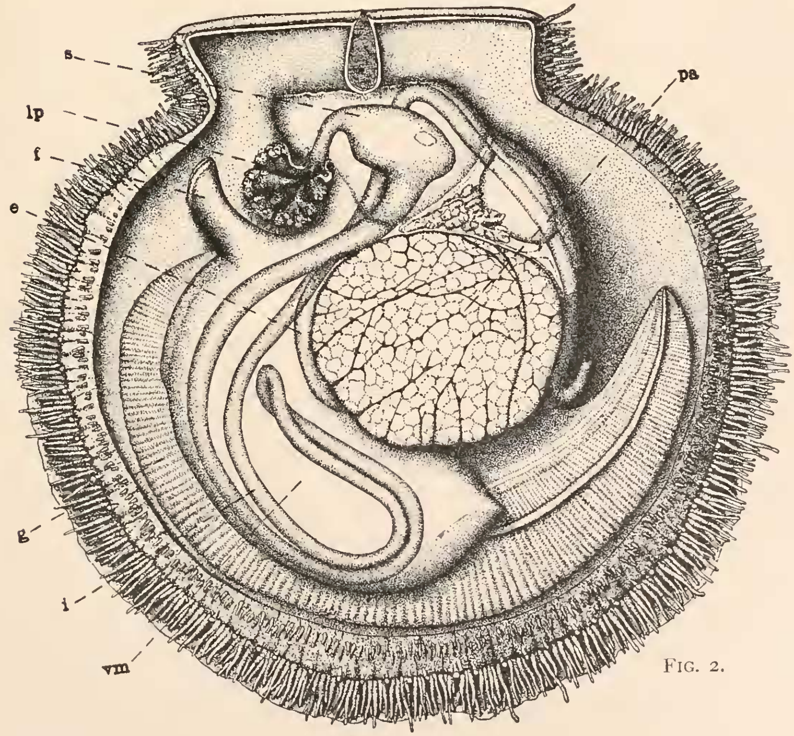

FIG. 2.

Drew del.

PLATE XVIII.

FIG. 3. Animal as seen from the left side with the left shell valve and mantle lobe removed. Drawn to show the arterial system of blood vessels. Two thirds natural size.

FIG. 4. Animal as seen from the left side with the left shell valve and mantle lobe removed. Drawn to show the systemic veins. Two thirds natural size.

a, auricle; *aa*, anterior aorta; *apa*, anterior pallial artery; *e*, excretory organ; *fa*, foot artery; *fv*, foot vein; *ha*, hepatic artery; *hv*, hepatic vein; *pa*, posterior adductor muscle; *paa*, posterior adductor artery; *pav*, posterior adductor vein; *ppa*, posterior pallial artery; *v*, ventricle; *va*, visceral arteries; *vm*, visceral mass.

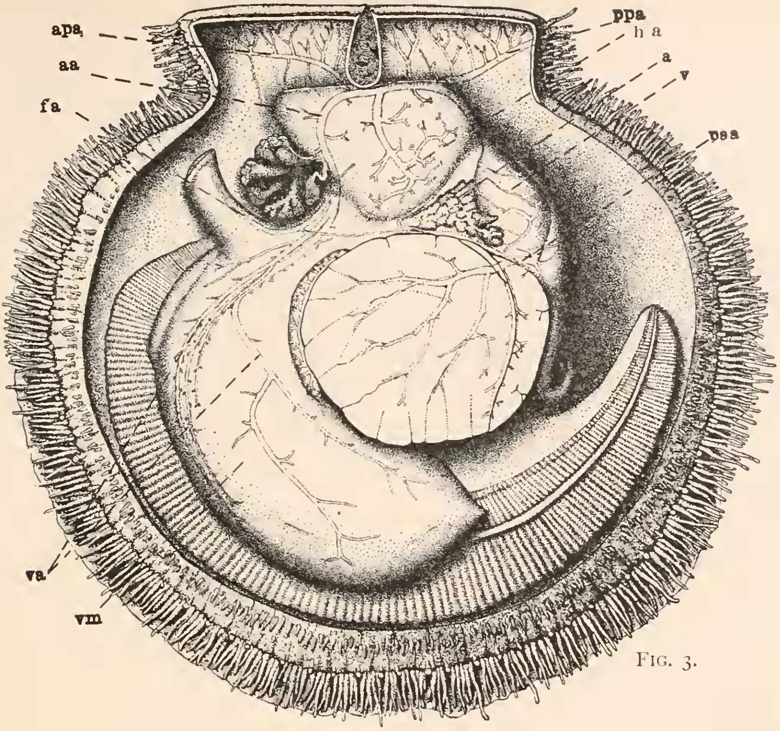

FIG. 3.

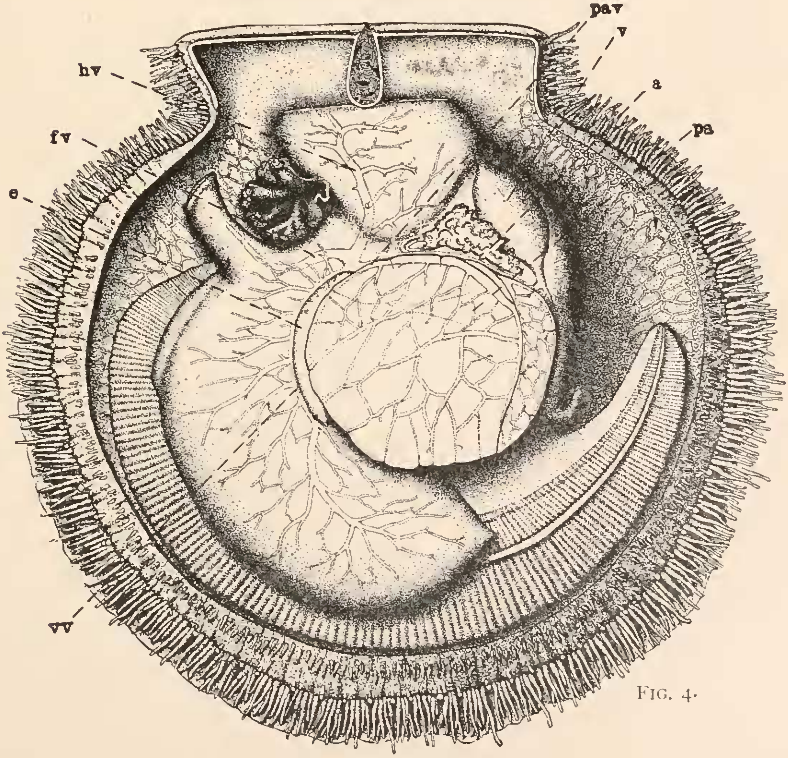

FIG. 4.

Drew del.

PLATE XIX.

FIG. 5. Outer surface of the left lobe of the mantle showing the arrangement of blood vessels. Two thirds natural size.

FIG. 6. Animal as seen from the left side with the left shell valve and mantle lobe removed. Drawn to show the nervous system. Two thirds natural size.

apa, anterior pallial artery; *apn*, anterior pallial nerve; *bn*, branchial nerve; *cc*, cerebral commissure; *cg*, cerebral ganglion; *cpn*, circumpallial nerve; *cvc*, cerebro-visceral connective; *ot*, otocyst; *pa*, posterior adductor muscle (anterior portion); *pa′*, posterior adductor muscle (posterior portion); *pg*, pedal ganglion; *ppa*, posterior pallial artery; *ppn*, posterior pallial nerve; *pv*, pallial vein; *vg*, visceral ganglion.

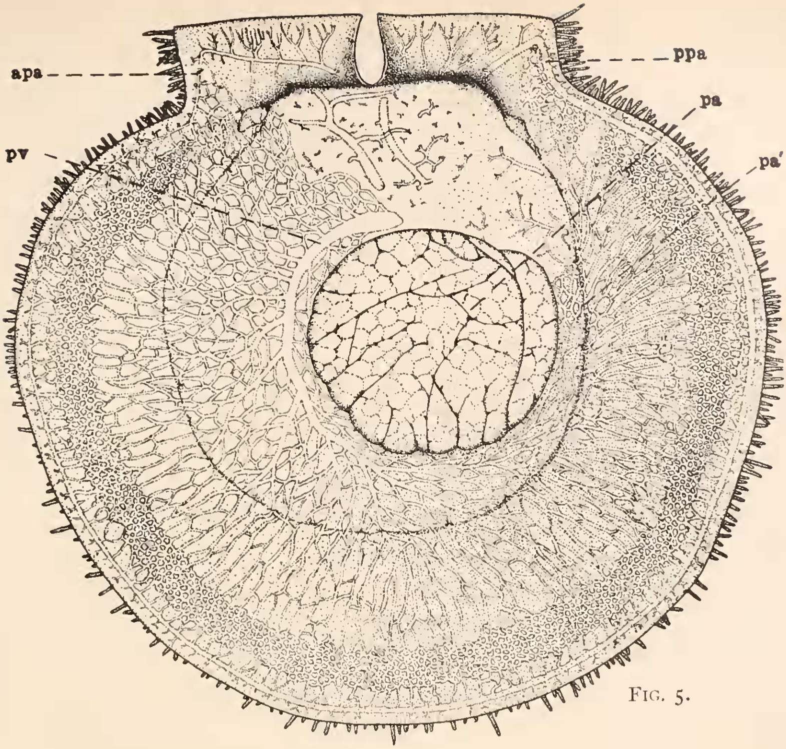

FIG. 5.

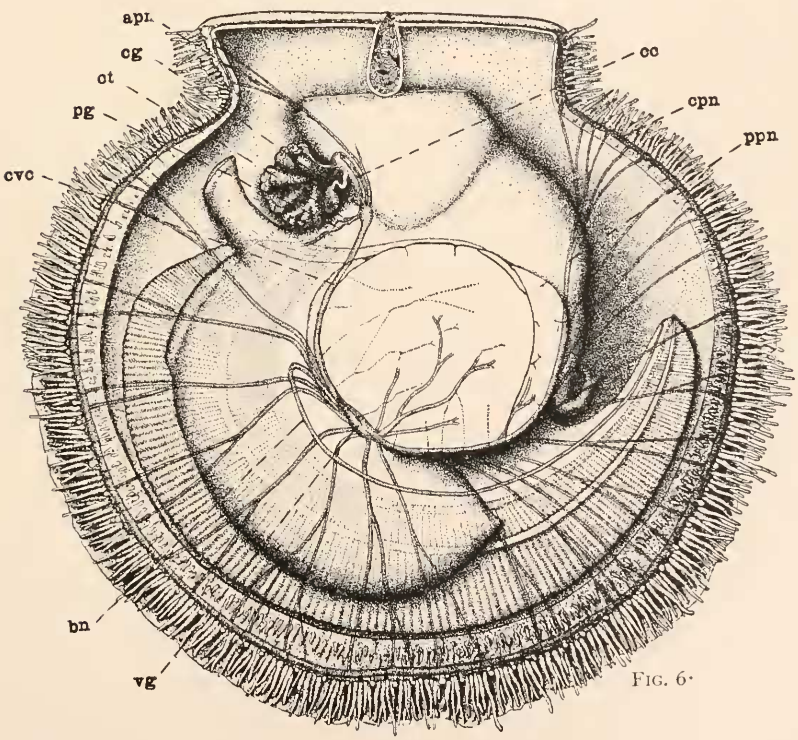

FIG. 6.

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PLATE XX.

FIG. 7. A portion of a gill showing the arrangement of parts. The figure indicates the inter-lamellar junctions cut at different levels. The further lamella is the one that was attached to the suspensory membrane and the vessel (*ba′*) was directly connected with the vessel that supplied the gill with blood (*ba*, Fig. 1). This vessel follows along the edge of the inter-lamellar junction to the free edge of the unattached lamella (the one on the side nearest the observer in the figure), where it bends back and passes down the modified filament as the vessel *ba′′*. Branches are given off from this vessel through the inter-filamentar junctions to supply the filaments. The vessel *bv′* is the vessel into which the blood that has traversed the gill is collected. It in turn communicates with the vein of the gill (*bv*, Fig. 1). Magnified about seventy diameters.

ba′, branch of the branchial artery; *ba′′*, branch of the branchial artery in the modified filament; *bv′*, branch of the branchial vein; *cr*, chitinous rod; *gf*, gill filament; *ifj*, inter-filamentar junction; *ilj*, inter-lamellar junction; *io*, inhalent ostium.

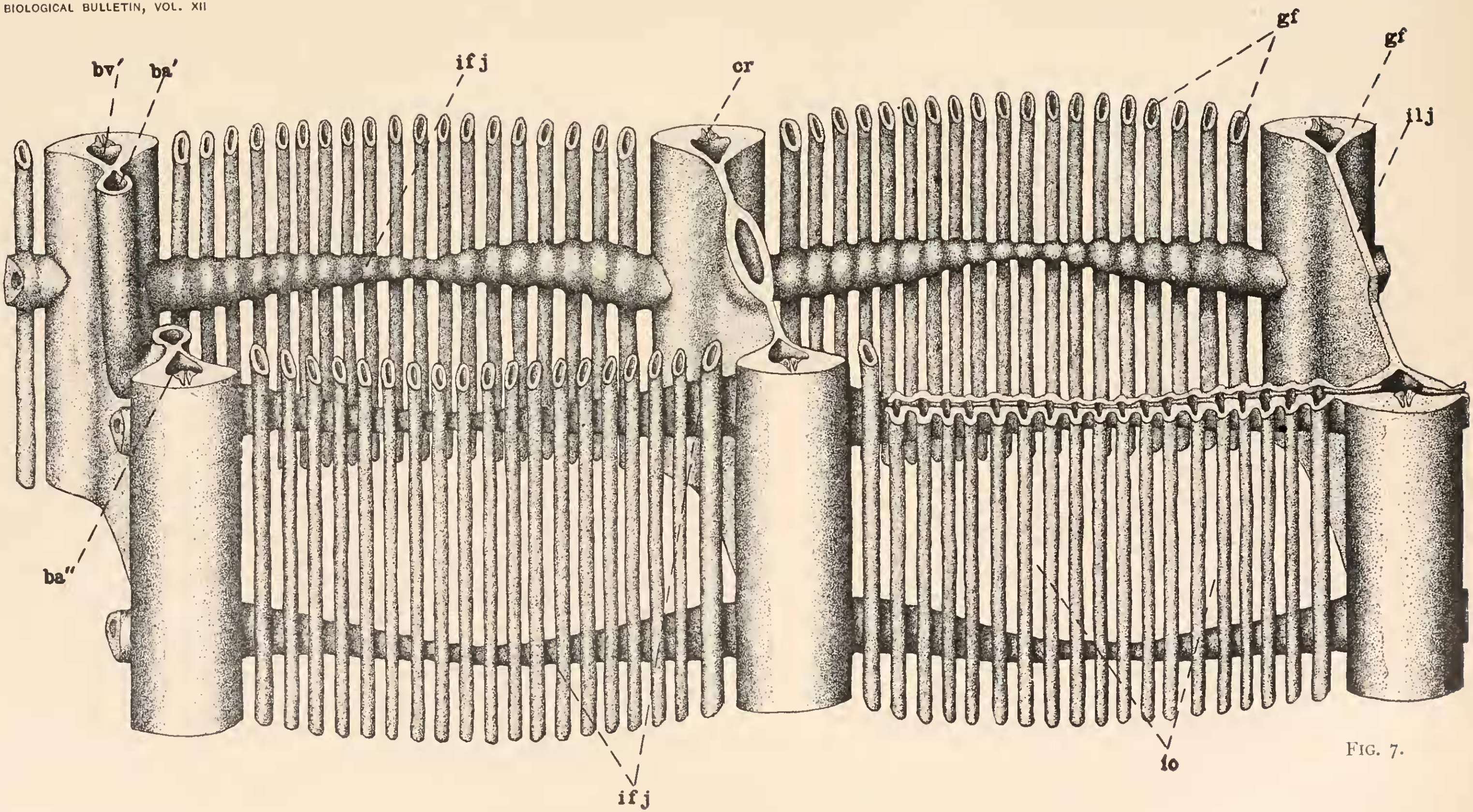

FIG. 7.

Drew del.

PLATE XXI.

Fig. 23. Nervous system as seen from in front and a little to one side. Natural size. (Diagrammatic.)

apn, anterior pallial nerve; *bn*, branchial nerve; *cc*, cerebral commissure; *cg*, cerebral ganglion; *cpc*, cerebro-pedal connective; *cpn*, circumpallial nerve; *cvc*, cerebro-visceral connective; *ot*, otocyst; *pg*, pedal ganglion; *pn*, palp nerve; *ppn*, posterior pallial nerve; *vg*, visceral ganglion.

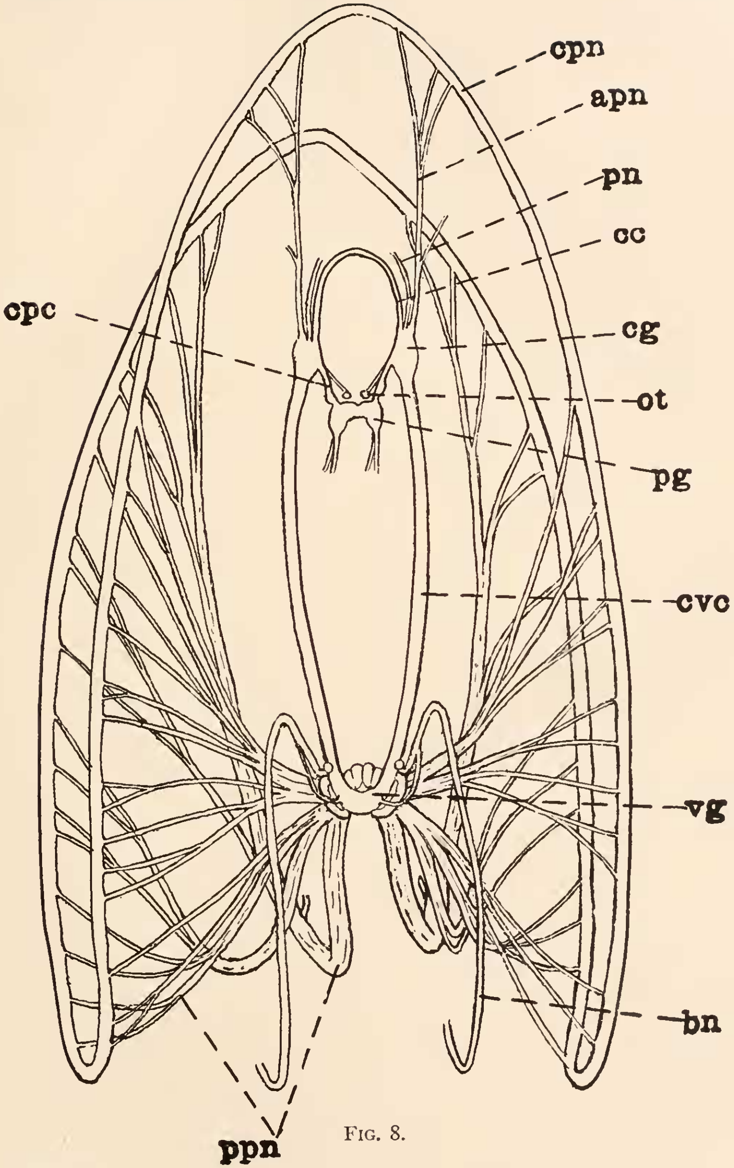

FIG. 8.

Drew del.

PLATE XXII.

Fig. 9. Cerebral and pedal ganglia with their nervous connections, as seen from the antero-ventral position. These ganglia and the otocysts lie in a mass of connective tissue and may be dissected out and mounted for study without injury Magnified about fifteen diameters.

Fig. 10. Visceral ganglia seen from the ventral side. These may easily be exposed for study by stripping the thin muscular covering from their ventral surfaces. They are hard to separate from the adductor muscle but they may be mounted with a thin piece of the muscle and studied in position. Magnified about fifteen diameters.

apn, anterior pallial nerve; *bn*, branchial nerve; *cc*, cerebral commissure; *cg*, cerebral ganglion; *cpc*, cerebro-pedal connective; *cvc*, cerebro-visceral connective; *fn*, foot nerve; *ot*, otocyst; *otc*, otocystic canal; *otn*, otocystic nerve; *pg*, pedal ganglion; *pn*, palp nerve; *ppn*, posterior pallial nerves; *x*, swelling on the visceral ganglion from which the anterior root of the branchial nerve originates; *y*, swelling on the visceral ganglion from which the posterior pallial nerves originate.

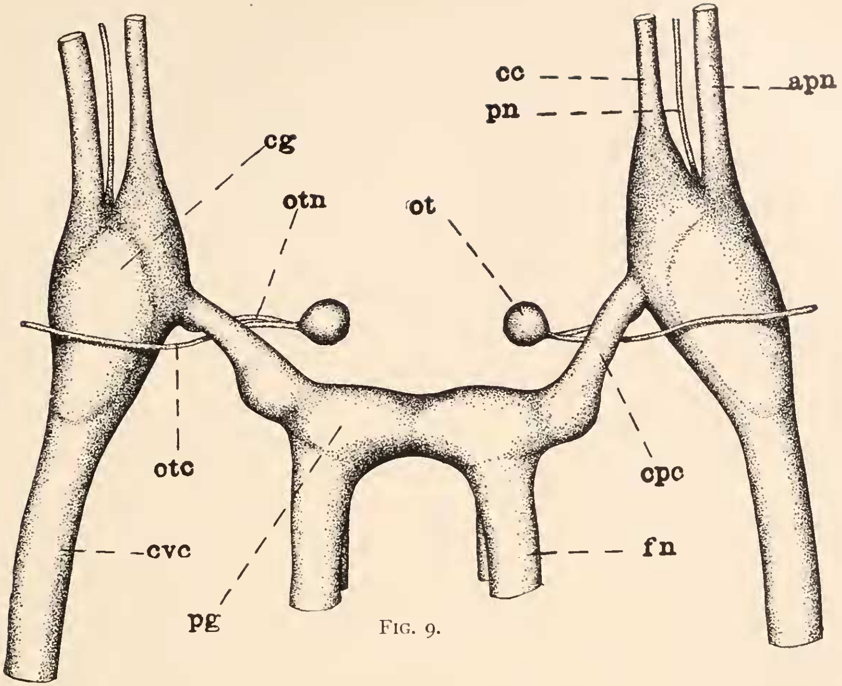

FIG. 9.

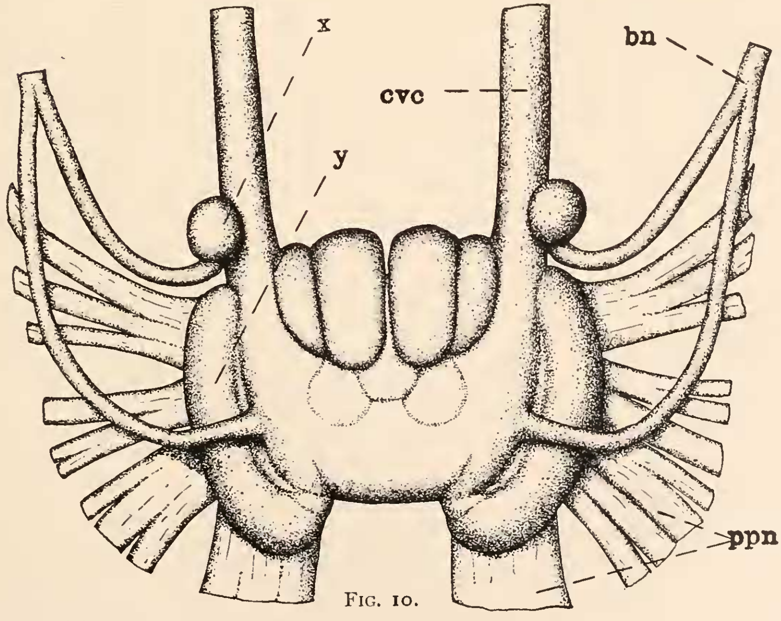

FIG. 10.

Drew del.

PLATE XXIII.

Fig. 11. Etching made from a photograph of the margin of Fig. 1, before that figure had been completed. It will be noticed that the same margin occurs on all of the figures that show this portion of the mantle.

Fig. 12. Etching made from a photograph of a combination of a photograph and an ink drawing. The photograph was made from Fig. 2 before the alimentary canal had been worked in. Fig. 2 was drawn on a print like Fig. 11. Figs. 3, 4 and 6 are etchings of drawings made by adding various organs on prints like Fig. 12.

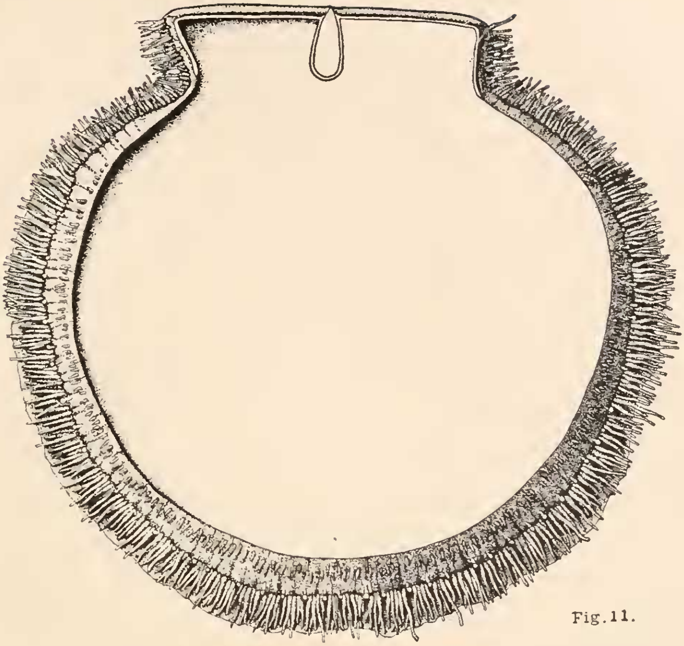

Fig. 11.

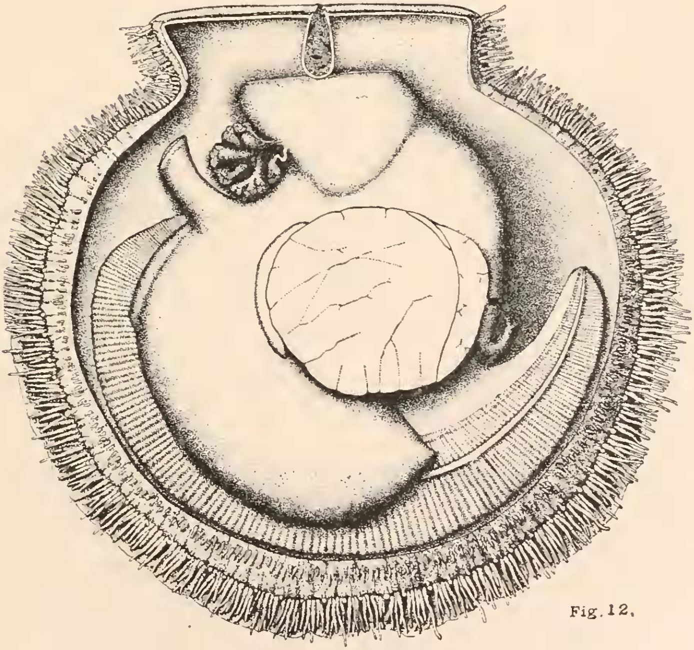

Fig. 12.

THE MATURATION OF THE MOUSE EGG.

WILLIAM B. KIRKHAM.

Sobotta ('95) after careful study of a very large number of preparations of the egg and ovary of the white mouse came to the conclusion that in nine tenths of these eggs the maturation processes involve the suppression of the first polar spindle, and the formation of only a single polar body. Gerlach ('06), after a study of preparations made at least as early as 1890, has revived Tafani's theory that in the majority of mouse eggs the second polar body is suppressed. Gerlach's conclusion is that when a spermatozoön enters an egg sometime after it has formed the second polar spindle, the second polar body fails to develop, and the spindle degenerates within the egg.

These results are at variance with the majority of opinions reached, before and since, by investigators of the eggs of other animals, vertebrate and invertebrate, and a reinvestigation of the maturation processes in the egg of the white mouse has brought it into line with most other metazoön eggs.

Material and Method. — The mice used have been killed during the period of most active breeding, namely, April, May, June and September, and serial sections made of the ovaries and Fallopian tubes. Ovulation, during the spring months, occurs very soon after parturition, independent of copulation, as observed by Rubaschkin ('05) in the guinea-pig.

When observed to be pregnant, the females were mated, and killed, some a few days or hours before parturition, others during that process, and still others at intervals from a few minutes to thirty hours after giving birth to a litter. The tissues were killed with a variety of the more generally used cytological fluids, and the following is a brief summary of the results obtained: All the ovaries contained some eggs with the second polar spindle and accompanied by the first polar body, and a majority of the series revealed ovarian eggs at the end of the spireme or with the first polar spindle. The eggs observed in the Fallopian tube fall into two main groups: those which had not been fertilized, and therefore retained the second polar spindle — some being accompanied by the first polar body, more without it — and those which had been fertilized. The latter included stages from the entrance of the spermatozoön through the cleavage stages.

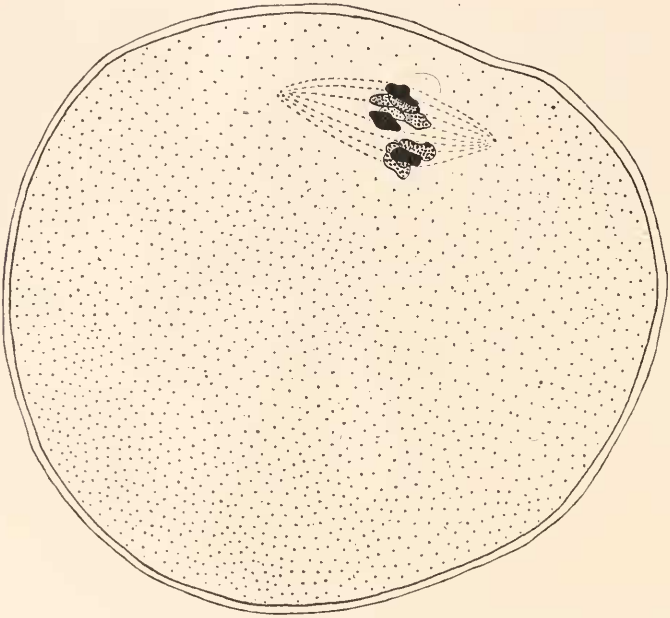

FIG. 1. Ovarian egg showing first polar spindle. Zona pellucida represented by double line. × 1200.

First Polar Spindle (Fig. 1). — The preparations in which there are stages immediately preceding the formation of the first polar spindle have not been fully studied, but there is evidence of a precocious division, the number of chromatin masses being between twelve and twenty-four.

The first polar spindle when first formed lies with its axis perpendicular to the radius of the egg, as found by Rubaschkin ('05) in the egg of the guinea-pig, and later one pole swings somewhat toward the center of the egg. The chromosomes of the first polar spindle are short and thick (Fig 2), and vary greatly in size. The spindle fibers come to more or less of a focus, and centrioles have often been seen at the poles of this spindle, where they are made up of several distinct, eccentrically placed granules.

First Polar Body (Figs. 3 and 5). — The study of many preparations reveals the following facts: None of the eggs in the Fallopian tube have failed to develop at least to the formation of the second polar spindle, and all the ovarian eggs which by their size, slightly denser protoplasm and large follicles appear to be nearly ripe, have already extruded the first polar body. The conclusion arrived at is, that apparently every egg which is capable of further development forms a first polar body within the ovary. This agrees with the observations of Rubaschkin ('05) upon the guinea-pig egg, and those of Van der Stricht ('01) upon the egg of a bat, *Vesperugo noctula*.

Fig. 2. Diagram of chromosomes in first polar spindle. Note great variation in size. Four more chromatin masses in adjacent sections.

This point established, it is next necessary to explain the disappearance of the first polar body in the majority of eggs seen in the Fallopian tube. The zona pellucida may persist in the mouse egg, undiminished, through the early cleavage stages, but in the majority of instances during the process of ovulation the first polar body is either forced through a weakened part of the zona, or frees itself by amœboid movements, and comes to lie outside the zona, as described and figured by Van der Stricht ('04).

The first polar body is usually oval in form, and is characterized, as found by Van der Stricht ('04) in the egg of *V. noctula*, by often possessing a little maturation spindle of its own, and in other instances having its chromosomes scattered. In some of these cases which possess a spindle, the first polar body would probably have divided mitotically, as observed by Sobotta ('95) in the mouse egg, and once by Rubaschkin ('05) in the egg of the guinea-pig. The polar bodies vary somewhat in size, and in one series of ovarian eggs there have been found first polar bodies of about four times the average volume. The number of chromosomes in the first polar body is twelve (dyads).

Second Polar Spindle (Fig. 3). — Immediately after the formation of the first polar body, the twelve dyads remaining in the egg are drawn into the equator of a new spindle, split longitudinally, and the twenty-four daughter, univalent chromosomes

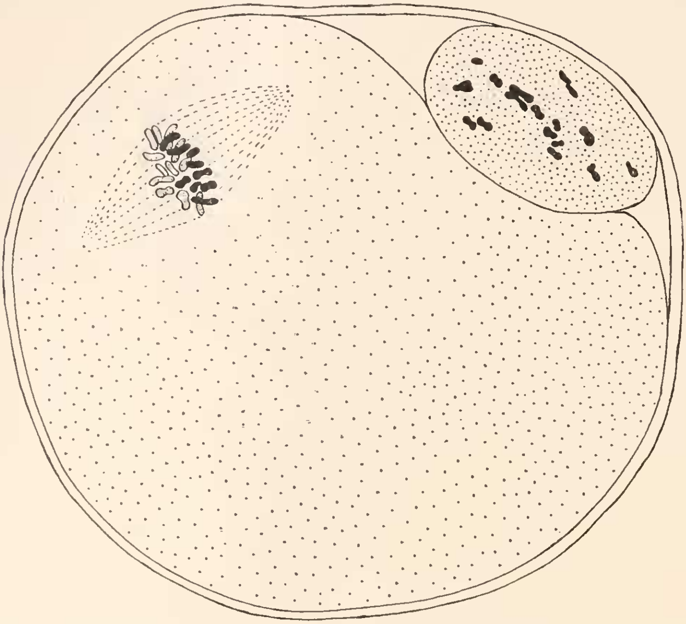

FIG. 3. Ovarian egg showing first polar body and second polar spindle. Seventeen masses of chromatin, some of which are undivided dyads, are scattered through the first polar body; twenty-four univalent chromosomes appear in the equator of the second polar spindle. Certain chromosomes have been added from adjacent sections. A minute centriole appears at each pole of the second spindle. The zona pellucida is represented by a double line. × 1200.

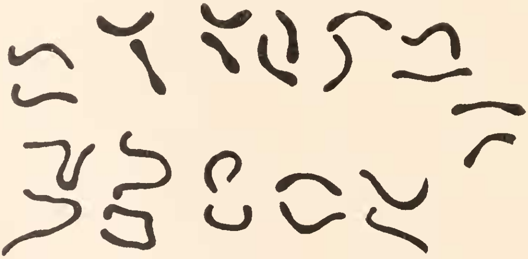

FIG. 4. Diagram of univalent chromosomes in second polar spindle, indicating difference in size.

lengthen out into filaments of various sizes (Fig. 4). Like the first polar spindle the second varies in size, and lies with its axis at right angles to the radius of the egg, usually near the first polar body. Centrioles, similar to those described above for the first polar spindle, have frequently been observed in second polar spindles, and in some cases a few radiating aster fibers have been seen at the poles. In attempting to determine whether a given polar spindle is first or second, the character of the chromatin has always been found a positive guide.

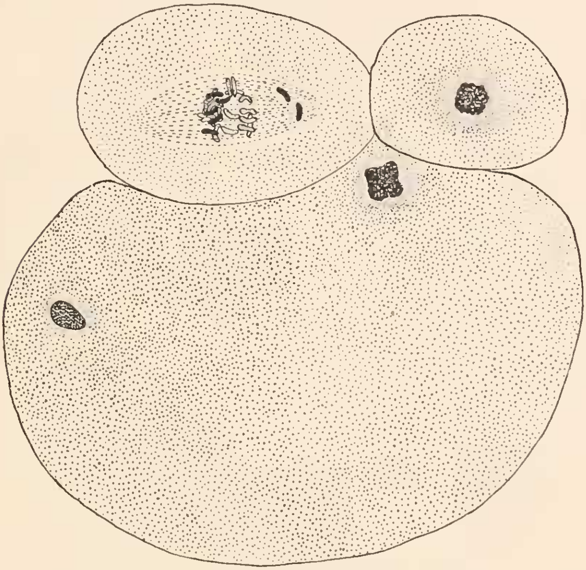

FIG. 5. Egg in Fallopian tube showing both polar bodies. Note spindle in first polar body. The sperm head appears at left, the female pronucleus at right, in the egg. × 1200.

Mature eggs which are retained within the ovary, together with such as are discharged and fail to be fertilized, degenerate with the second polar spindle, as found by Rubaschkin ('05) in the case of the guinea-pig egg.

Second Polar Body (Figs. 5 and 6). — Only one spermatozoön enters an egg, and it carries in most, if not all of its tail, a fact observed by Van der Stricht ('04) in the egg of *V. noctula*. When fertilized the egg at once forms its second polar body. This is more or less nearly spherical, smaller than the first polar body, and, as stated by Van der Stricht ('04) for *V. noctula*, generally has its chromosomes gathered into a single compact mass. It quickly forms a resting nucleus, possessing compact masses of chromatin, and is usually the only polar body seen during the early cleavage stages. In one instance (Fig. 6.) a second polar body was observed which had just been constricted off, and in consequence showed the separate chromosomes, twelve in number, and another preparation showed the second polar body forming the resting nucleus.

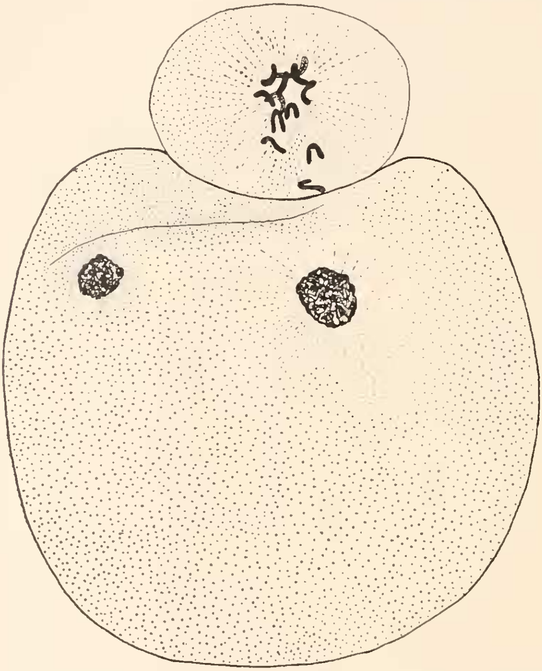

FIG. 6. Egg in Fallopian tube showing second polar body. First polar body has disappeared. At left in the egg is seen the sperm nucleus, and above it the separated tail of the spermatozoön; at right appears the egg nucleus, surrounded by delicate radiating fibers. × 1200.

The mouse egg is thus shown to be no exception to the general rule, that the maturation process in the metazoön egg involves the formation of two polar bodies.

In closing, I desire to express my gratitude, and great indebtedness to Professor Wesley R. Coe for his constant oversight and encouragement.

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ON THE ZOÖLOGICAL POSITION OF THE ALBINO RAT.[1]

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According to Leunis ('83) the black rat (*Mus rattus*) was known in Europe as early as the twelfth century, while the Encyclopedia Britannica (Olfield Thomas, '86) states the appearance of the black rat to be at least as early as the thirteenth century. Although the statements by the different writers as to the appearance of the black rat in Europe do not quite agree, yet it is clear that the arrival of the black rat was much earlier than that of the brown rat (*Mus norvegicus*)[2] which, according to various records, appeared in Europe at about the middle of the eighteenth century, or a little earlier.

Although both species of rats are described as originally natives of Central Asia, yet they are everywhere enemies. By the incessant competition between these two forms, the black rats were almost exterminated, first from Europe, and later from the greater part of North America, and at the end of the eighteenth century, the brown rats were alone found in abundance in these regions.

It is often stated that the white rat at present found in captivity, is the albino of *Mus rattus*. In support of this view there are a number of statements to be found in the older literature (Donndorff, 1792). (No effort has been made to examine the records previous to Linneus).

It is apparently on the basis of these records in the older literature that the current statements in popular natural histories and in encyclopedias are based.

On the other hand, in the zoölogical literature in the nineteenth century, there are numerous statements which refer to the albino rats as a variety of *Mus decumanus*.

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[1] From the Wistar Institute of Anatomy and Biology at Philadelphia.

[2] *Mus norvegicus*, Erxleben = *Mus decumanus* Pall. of older Zoölogical Literature. *Norvegicus* has priority, and has come into general use within the last two or three years.

Von Fischer ('69) in a catalogue of the mammals of the St. Petersburg Government, makes the following statement:

"Die Wanderratte, *Mus decumanus* Pall. (russisch Krýssa — Krýssa heist eigentlich *Mus rattus*, diese art ist bekannt unter dem namen Passjúck) kommt ueberall massenhaft vor in allen Farben; schwartz, schmutziggrau bis rostgelb, weissgescheckt und auch ganz weiss. "Die Hausratte, *Mus rattus* L., habe ich nie gefangen, weshalb ich annehmen zu durfen glaube, dass diesse Ratte hier auch nicht vorkommt."

Von Fischer ('74) used a white *Mus norvegicus* in his experiments on the production of hybrids. Later Crampe ('85) also used a white *Mus norvegicus* in experiments of the same nature.

Haacke ('95) and Bateson ('03) studied the crosses between the white *Mus norvegicus* and the common brown rat. None of the authors, however, describe in detail the white forms which they employed.

Despite the general belief to the contrary, there are many reports in recent literature indicating that groups of *Mus rattus* are still to be found in a number of localities, both in Europe and the United States.

In the United States, *Mus rattus* is reported from Texas, Florida and other southern states, and also from Iowa. Rhoads ('03) reports a number of new localities in the States of Pennsylvania and New Jersey. It has been learned through Director Dr. Seitz that in Germany the black rat is present in large numbers in the buildings connected with the zoölogical garden in Frankfurt a/m.

It may be interesting to note that the occurrence of white rats in a wild state has been reported from two localities in Iowa, by students working in the neurological laboratory at the University of Chicago. There are no means of determining, however, whether these were albinos of the black or brown rat. From this review it is evident, therefore, that there are, or have been, at least two forms of albino rats.

Since 1893 a colony of albino rats has been maintained in the neurological laboratory at the University of Chicago, and in 1906 a similar colony was established at the Wistar Institute of Anatomy at Philadelphia.

These colonies have been recruited for the most part from the northern states of the Atlantic seaboard, but some specimens have come from as far south as Missouri. All the rats received from these various localities have appeared to be of the same variety, and have always bred true.

Heretofore, the specific similarity of the albinos and the other forms has been concluded from observation of the external characters only. Wishing more exact information as to the zoölogical relation of the rats composing these colonies, the present investigation was undertaken to determine whether we were dealing with an albino variety of *Mus rattus* or *Mus decumanus.*

Externally, *Mus rattus* is usually distinguished from *Mus norvegicus* by the following specific characters:

Mus rattus is smaller in size. The tail of *Mus rattus* is considerably longer than the body, while in *Mus norvegicus* it is either shorter or only slightly longer than the body, but not relatively as long as that of *Mus rattus.*

The following measurements, though incomplete, serve to indicate this relation:

Table Showing Length of Boby and of Tail.

Observer.	*Mus rattus.*			*Mus norvegicus.*		
	Length Body.	Length Tail.	No. of Obser.	No. of Obser.	Length Tail.	Length Body.
New International Encycl.	21 cm.					27 cm.
Leunis	16 cm.	19 cm.			19 cm.	24 cm.
Hatai				27 males	21 cm.	24 cm.

The general shape of the head (see Fig. 1) of *Mus rattus* is slender, the nose is sharper, and the ear is both wider and longer than in *Mus norvegicus.* It may be worth while to mention that the so-called Alexandrian rat (*Mus alexandrinus*) is said to have external characters similar to those of the black rat (*Mus rattus*) and these two species are only distinguished by their coloring, *Mus alexandrinus* having a brown colored coat.

If we compare the external bodily characters of the albino rat found in our rat colonies, with those of the brown rat, we are surprised by their close similarity. All these characters of the brown rat are also characters of the albino rats composing our colonies. In other words, the common brown and our albino rats cannot be distinguished from one another by their external characters.

It is nevertheless true that the albino rats which we have examined, are smaller in size than the brown rats in the same localities. In fact, the absolute size of the albino rat is nearly intermediate between *Mus rattus* and *Mus norvegicus*. It is possible that the confinement in which these albinos have been reared, accounts for their smaller size, as the result of lack of exercise and altered conditions of life. It is possible also that we have here a phenomenon similar to that described by Semper ('81) and De Varigny ('94) on snails, where the size of the animals diminished with the size of the vessels in which they were reared.

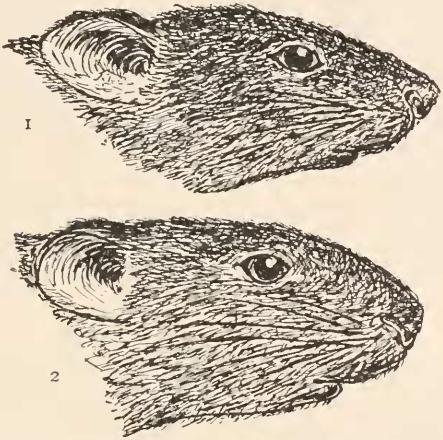

FIG. 1. Copied from "Encyclopedia Britannica," in order to show the shape of the heads of the brown and black rats. 1. *Mus rattus*. 2. *Mus norvegicus*.

It was thought that the character of the skull might serve for a more exact distinction of the forms under discussion. We therefore examined and compared the skulls of *Mus rattus*, *Mus norvegicus*, and of the albinos.[1]

[1] In order to make this comparison, it was necessary to examine as many skulls as possible, and I am indebted to Professor J. A. Allen, American Museum of Natural History, at New York, Professor Elliot, Field Columbian Museum at Chicago, Dr. Greenman, The Wistar Institute of Anatomy at Philadelphia, and Professor Merriam, National Museum at Washington, for putting at my disposal various series of skulls, possessed by their several institutions.

To illustrate the differences found, both photographs and drawings have been made.

On comparing the skull of *Mus rattus* with the brown rat, the general unlikeness can be seen in Fig. 2.[1] The most noticeable difference is in the shape of the cranium.

When viewed from the dorsal aspect, the cranium of *Mus rattus* is oval in the outline, while that of *Mus norvegicus* is somewhat rectangular. Moreover, the dorsal aspect of the cranium in *Mus rattus* is decidedly convex, while in *Mus norvegicus* it is nearly flat. In *Mus rattus* the os nasale as compared to the entire length of the skull, is relatively shorter than *Mus norvegicus.* In *Mus rattus*, the outline of the os interparietale is somewhat semilunar in shape, while in *Mus decumanus* it is rectangular. In *Mus rattus*, the os parietale is broader as compared with its length, than in *Mus decumanus.* In *Mus rattus*, the foramen magnum is subcircular in outline, while in *Mus norvegicus* it is somewhat rectangular. On the ventral aspect of the skull, the large tympanic bullæ in *Mus rattus* are more conspicuous and eminent than in *Mus norvegicus.*

The junction point of the os basi-sphenoidale and os basi-occipitale is flat in *Mus rattus*, and protrudes in *Mus norvegicus.* The anterior end of the maxilla which forms the lateral wall of the infraorbital fissure, is blunter in *Mus rattus*, than in *Mus norvegicus.* The skulls of our albino rats are very similar in the above characters to those of *Mus norvegicus*, and the description of *Mus norvegicus* may be taken to apply to them.

In connection with the shape of the skulls, the determination of a cranial index has been made. The index used, was that obtained by dividing maximum width of the cranium by the length of the fronto-occipital line. (See Fig. 3.) On account of the small number of specimens measured, the accompanying table is to be considered as merely preliminary, but as it stands it shows a similarity in this index between *Mus norvegicus* and the albino rats, and a difference between these two forms and *Mus rattus.* The cranial index will be made the object of a more extended investigation.

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[1] Care has been taken to use only the skulls of fully matured animals. See J. A. Allen ('94) and H. C. Merriam ('95).

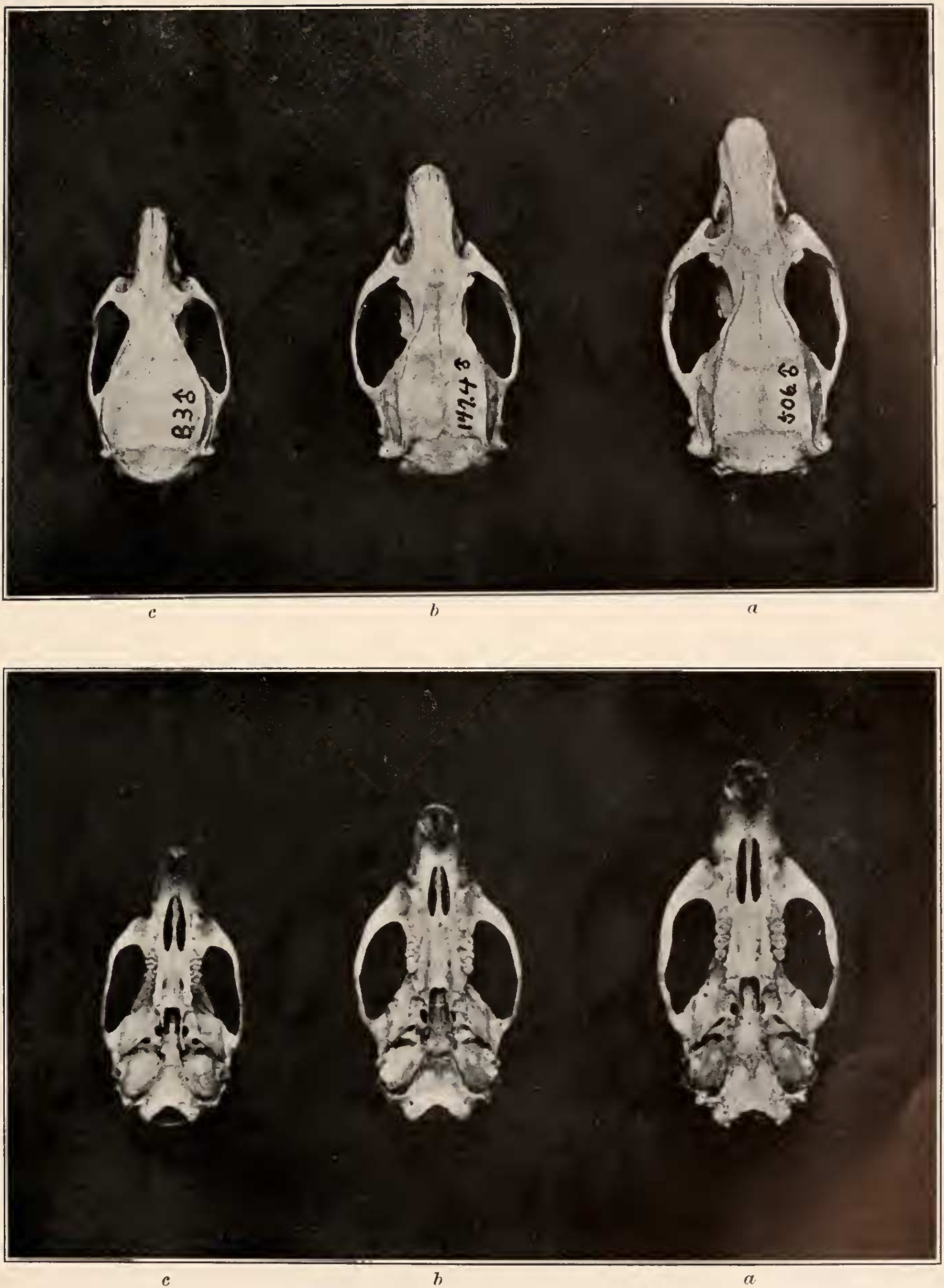

FIG. 2. Shows the skulls of *Mus norvegicus* (*a*), albino rat (*b*) and *Mus rattus* (*c*). The skulls were photographed from two different aspects, in order to show various views of the skulls for a comparison.

The upper row was taken from the dorsal aspect, and the lower from the ventral. The figures are about the natural size.

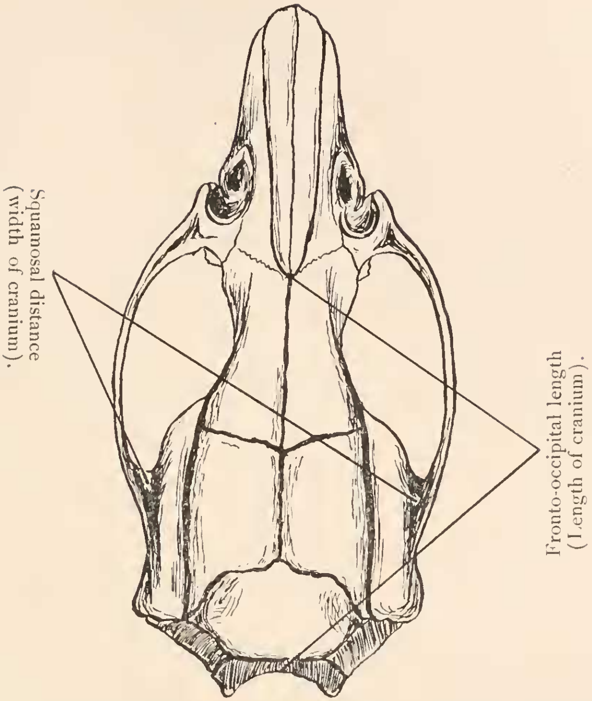

FIG. 3. (× two diameters.) The measurement of frontal-occipital length was determined in the following way:

Since the length measured from the tip of the nose to the posterior end of the inter-parietal bone, is not always equal to the length measured from the tip of the nose to the end of the occipital bone, both measurements were taken. First, the measurement from the tip of the nose to the end of the occipital bone, and second, that from the tip of the nose to the end of the inter-parietal bone. The difference thus obtained, was added to the length of the frontal-interparietal line, and the sum was called frontal-occipital length.

The width of the cranium was determined by taking a maximum width between the two points (right and left) where the zygomatic bones rest on the lateral walls of the cranium.

We conclude therefore, that the albino rats composing the colonies at Chicago and Philadelphia, are similar to *Mus norvegicus* in their bodily proportion, and in their cranial characters. They are however, smaller in size than the specimens of *Mus norvegicus* usually found.

TABLE SHOWING CRANIAL INDEX.

Males.	Cranial Index Average.	Extremes.	No. of Rats Used.
Mus rattus	.60	.58–.62	8
Mus norvegicus	.54	.51–.55	12
Albino rat	.54	.50–.56	12

Nevertheless this form is to be regarded as an albino variety of that species and to be designated *Mus norvegicus* var. *albus* (*oculis rubicundis*).

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NOTES ON THE BEHAVIOR OF SEA-ANEMONES.[1]

CHAS. W. HARGITT.

During the summer of 1901 while keeping a few sea-anemones in the aquarium for the purpose of studying their general habits, particularly those of feeding, my attention was drawn to the interesting phenomenon that certain species appeared more alert during the night, closing up more or less during the day. This was more noticeable in the large sand-anemone, *Eloactis producta*, whose peculiar habit of burrowing in the sand, enabled it to withdraw entirely when disturbed, or under other unfavorable conditions.

Having secured several specimens of this anemone they were placed in an aquarium, the bottom of which had been covered with sand to the depth of some six inches or more. The specimens, true to their habit, soon burrowed deeply in the sand, and lining the burrows with a slimy excretion they soon seemed quite at home. During the day they would be found with only the whorl of tentacles quietly protruding at the surface of the burrow, where their colors so closely conformed to that of the sand that the casual observer would hardly notice their presence. Going into the laboratory at night I was interested to see the specimens greatly extended, half of the body protruding beyond the burrows and tentacles raised in an attitude to seize passing prey. This was frequently observed afterward, and notes made of it at the time were recorded in which it was remarked that "these creatures are probably nocturnal in their habits."

At the same time I had under observation another anemone, *Sagartia leucolena*, a very common species about Woods Holl, and it was seen to migrate at times into darker portions of the aquarium, even creeping under bits of rock or other objects.

No further observations were made on the subject till the current summer. About a dozen specimens of *Eloactis* were collected and placed in the aquarium as before, and with the same

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[1] Contributions from the Zoölogical Laboratory, Syracuse University.

result that such specimens as found sand proceeded to cover themselves as far as possible. In the light of current activity and interest in the matter of behavior it occurred to me to subject these creatures to a few experiments with a view of testing their reaction to light, and perhaps a few other environmental factors.

An examination of the available literature has brought to light but few instances in which any observations have been made concerning the behavior of actinians in relation to light. The Hertwig brothers, '79 (" Die Actinien Anatomisch und Histologisch," p. 191), cite brief observations made by Quatrefages on species of *Edwardsia* in 1842, and by Haime on *Cerianthus* in 1854, and include likewise brief references to their own observations on a deep sea-anemone, *Cladactis costæ*, in the Naples aquarium.

Quatrefages found that when a ray of light from a lamp was condensed upon the specimens by means of a lens they partially retracted. Haime observed that in bright sunlight species of *Cerianthus* contracted within their tubes and later expanded when the light became less intense. The Hertwigs record simply the fact the specimens during full daylight were more or less contracted and expanded as the light became less intense. " Im tageslicht zieht sie ihren Korper stark zusammen und erst wenn es zu dunkeln beginnt, dehnt sie sich auf das Vier- bis Funffache aus und entfaltet ihre Tentakeln, di zuvor eingezogen waren."

Jourdan has recorded a similar observation (" Les Sens chez Les Animaux Inferieurs," Paris, 1889), made upon a species of *Paractis* in which similar behavior was exhibited. " J'ai pu voir moi-même, sur des Actinies du genre Paractis, des manifestions evidentes de cette sensibilité spéciale. Des Orties de mer restent fermées aussi longtemps qu'on les expose à une lumière trop vive ; alles ne o'epanouissent que lorsqu'on les met à l'abri des rayons lumineux" (p. 221).

Eloactis producta. — My first observations were made to confirm those already cited, namely, to clearly demonstrate their nocturnal habit. Placed in the aquaria of the general laboratory, and in a few cases in smaller jars in my private laboratory, their behavior was closely watched after the specimens had become adjusted to their new habitat. In this connection should be mentioned the fact, to be discussed later, that some specimens were much less prompt in burrowing, a few remaining more or less indifferently upon the surface of the sand and showing but slight attempts to bury themselves.

It only required a few observations to determine beyond any doubt that only in light of low intensity, such as twilight, or in the aquarium under the rather dim light of an incandescent lamp at some distance, did the specimens protrude their oral portions and tentacles and show any degree of activity. To further demonstrate that these seemingly nocturnal activities were not merely a periodic response made at more or less definite intervals, the following experiment was made. A tall glass jar, some twenty inches in depth, the lower third of which was filled with sand, in which had been placed several anemones some two days previous, was so placed on a laboratory table that it was freely exposed to the diffused light of the room. Over the jar was placed about mid forenoon, when the creatures were securely withdrawn in the burrows, a blackened chamber or dark hood, so arranged as to exclude more or less perfectly the light. Removing the hood at the end of an hour it was found that the creatures were quite extended as at night. And it was soon evident, that with the removal of the hood and the admission of light, they were at once aware of the change and promptly began to show signs of irritation, which ended within five minutes in every specimen having retracted into its tube. To make certain that the response had not been induced by some mechanical stimulus, such as the tremor of passing steps, or an accidental disturbance of the table or the water in the jar, the experiment was repeated within a half hour and under conditions which made it possible to observe the phases of the response.

Within fifteen minutes after the chamber had been placed over the jar it became quite evident that the change had been recognized by the specimens. This was shown first by the extension of the tentacles, and next by a slow protrusion of the oral region by degrees, till within about half an hour the body was extended an inch beyond the surface, as before. Again removing the chamber and thus exposing the specimens to the light, within two minutes, indeed, almost immediately, they began to retract. This reaction is not sudden or general at once, as in such creatures as the earthworm, but begins in a somewhat indefinite movement of the body, accompanied by similar movements of the tentacles, followed very soon by a slow but definite retraction of the entire body within the tube, often including likewise the tentacles as well.

The experiment was later repeated in a room where it was possible to utilize direct sunlight. Under these conditions the reaction was much more energetic and definite, as might be expected. Variously modified, the experiments were performed repeatedly, perhaps fifty times, and with substantially the same results, though, as will be noted in a later connection, exhibiting variations of response. In some cases the reaction was so definite and prompt as to leave the impression on the observer that the creature was possessed of something akin to visual sensation. At other times the reactions were indefinite, sluggish, variable, and less convincing, though in the end resulting in the retraction of the specimen as before.

The following experiment was made to determine the extent of the sensory area, or in other words whether all portions of the body were similarly responsive to light. A specimen which had been quietly expanded on the surface of the sand for some time, being one of those which had shown less aptitude for burrowing, was so placed as to make it possible to reflect a narrow ray of light upon sharply defined parts of the body or tentacles. It was found that the oral region, including about one third of the body, was distinctly more sensitive than was any other. Light concentrated on the aboral portion seemed to have no effect at all, or so slight as to be indistinguishable. The tentacles were apparently less responsive than the immediately adjacent oral part of the body. This is slightly different from the condition found in *Sagartia modesta*, as will be noted later, and was a matter of some surprise, since the pigmentation of the tips of these organs might be thought to have some relation to sensory functions.

In a general way these results confirm the histological studies of the Hertwigs (*op. cit.*, p. 22), as to the distribution of the sense cells in actinians. They also agree substantially with some of their experimental observations as to the unequal distribution of the sensory areas, though on this point they gave slight attention to the effect of light as a stimulus (*ibid.*, p. 190).

Sagartia modesta. — This anemone has much in common with the former species. It is a creature having its habitat in the sand just below or near low tide line. Like the former it takes somewhat readily to the artificial environment of the aquarium, though seems somewhat less hardy under these conditions. I first studied this species in its native haunt, having found several specimens on an accessible beach. I first found them just before twilight, and in the shadow of a large boulder which still further reduced the light, with the tentacles extended very much as in the case of *Eloactis;* the body was not protruded beyond the tube. Going again in the brightness of early morning they were not to be seen, no sign of tentacles even in the partially closed burrow. I made these observations several times, and concluded that they were probably also nocturnal.

Specimens were collected and taken to the laboratory and placed in the same general conditions as were the former species. Experiments similar to the former were performed, but with much less promptness or clearness in reactions. Placed under the dark chamber there was not the ready extension of the body as in *Eloactis*. Further, on removal of the hood the response was much less sharp and convincing, though quite evident. Placed on a table upon which a beam of sunlight could be reflected it was found when the ray was reflected upon the numerous tentacles that there was immediate reaction. It should be stated that in this species the tentacles are very numerous, even a hundred or more, and form a dense crown in expansion covering the oral region like an umbrella, while in the former species these organs are but twenty in number and rather short. In *Segartia* the tentacles seemed more sensitive than in the former species, or than the oral region, but this may be due in some measure to their numbers, and to the general relations they sustain to the oral portion of the body, especially the region just below the tentacles. Still the results agree again with the views of the Hertwigs, as expressed in the following words: "Die Sinneszellen finden sich im Ektoderm der Mundscheibe und der Tentakeln, wie uns schien, überall ziemlich gleichmässig vor; nur an der Spitze der Tentakeln mochten sie vielleicht in grösserer Anzahl vorhanden sein" (*op. cit.*, p. 22).

Similar experiments were made on three other species of anemones, namely, *Sagartia leucolena*, *Sagartia luciæ*, and *Metridium marginatum*. These species are all more or less free, and variable as to habitat. The first, *S. leucolena*, is fairly common at various points along the shore-lines of the region of Vineyard Sound and southward. Its usual habitat seems to be under rocks near low tide, though taken also on the piles of docks. It seems to seek the under sides of rocks, or settles among masses of *Molgula*, sponges, etc., on piles, thus more or less secluded, and seldom seen by the casual observer.

On the other hand, *S. luciæ* seems to be equally at home almost anywhere in shallower pools, on fucus, piles of docks, etc., sometimes in shaded places, but oftener in the open sunlight on rocks, fucus, etc. About the same may be said of *Metridium*. While more common from deeper water than either of the others, it is yet quite common just below tide line on rocks, piles, etc.

The experiments on these species were made under the environment of the aquarium, but were sufficiently varied to give fairly satisfactory tests as to their reactions to this class of stimuli.

From what has been said as to the habitat just given it might be inferred that *Sagartia leucolena* would prove the more responsive to the tests, and such was found to be the case without exception, though as in the former cases, with considerable individual differences.

Verrill long ago pointed out that this species was more active when in dimly lighted aquaria, or at night. However, I have not found that specimens in the general light of the laboratory showed any very evident light reactions. But when an aquarium was placed in direct sunlight there was an almost uniform attempt on the part of specimens to escape from the direct rays. As a rule this was done by slowly creeping over the edge of the stone or shell into a less exposed position. Specimens which were in glass jars, and attached to the sides or bottoms of the jars, when brought into direct sunlight soon closed up entirely, withdrawing even the tentacles, and assuming a more or less hemispherical shape. Taken from the direct light into the diffused light of the room they promptly expanded and remained so until again placed in the sunlight. This experiment was repeated again and again, and with substantially the same results. It was also found that the degree of contraction was very closely an expression of the degree of light intensity.

Many specimens were brought to the laboratory adhering to small rock fragments, bits of shells, etc. In a few cases when such specimens were exposed to direct light they would creep over to the shaded side of the rock, and during the night return to apparently the exact spot previously occupied. This might be taken to suggest some such sense of position as is known to be had by certain gasteropods; but the tests were not sufficiently numerous nor constant to warrant any definite statement.

With *Sagartia luciæ* and *Metridium* the case was very different, as might be expected. Specimens of these anemones placed under the same conditions as the former, indeed in many cases when occupying the same aquarium, were found to be almost without exception, quite indifferent to light. Placed for some time under a dark hood and suddenly exposed to direct sunlight there was not the slightest evidence that there was any sense of the change. The experiment was made in various ways. Sometimes as just suggested. Again, a beam of strong light was reflected directly on the specimen as it was quietly expanded on the table, but so far as *S. luciæ* was concerned, always and without exception, with negative results. Occasionally, though always doubtfully, *Metridium* would show some slight sensory movements of the tentacles. But specimens have been subjected to the reflection of a strong beam of light directly upon the oral surface for ten minutes at a time without the slightest response.

I have had a few specimens of *Edwardsia elegans* in the aquarium but for some reasons they did not seem at ease under these conditions, and exhibited no distinct evidence of any photic sensibility. I have seen but once any living specimens of *Cerianthus* at Woods Holl and then only under circumstances which rendered any observations impracticable. I regret therefore, not to have been able to test the sensory behavior of these species.

The only other aspects of behavior which have been observed are those of feeding, and the very variable reactions concerned in tube-building.

Concerning the former my first experiments were made several years since. At that time I tested their feeding propensities by trying in various ways to induce them to take food. At various times during their aquarium life I tried to feed the creatures with bits of crab meat, bits of fish, clam, etc., but in no case was I able to induce the creatures to take the bait. During the present summer I observed that specimens of *Eloactis* which had been dug up and placed in a pail along with specimens of *Balanoglossus* were found devouring the latter alive. This was so unlike the former behavior that one was tempted to wonder whether they might have peculiarities of diet, and that their habitat on these sand flats, where likewise *Balanoglossus* has its home, might sustain some relation thereto. I therefore repeated the former experiment of offering them shreds of crab and fish meat and with the same negative results. I then tested them again with the *Balanoglossus* and found that it was taken quite readily by the same specimens which had refused the other bait. Leaving them for several days they were again tested with the same foods and with the same negative results. Having no specimens of *Balanoglossus* at hand some annelids, *Hydroides*, were offered them alive, and they were readily taken by three out of four tested.

No further qualitative tests were made along this line, but it would seem as if they were rather partial as to feeding habits, and particularly as to whether it be living or otherwise.

Limited tests were made as to their reaction to such substances as blood of crabs, clams, etc., but there seemed hardly any definite reactions indicative of olfactory, or gustatory sensibility. The swallowing reaction of *Eloactis* is much as in other species of actinians, namely that it consists largely of oral efforts. The tentacles play but little part in the reaction, though serving to press the food down upon the oral margins or lips. The swallowing act in these creatures involves something of a peristalsis of the esophagus. It was observed in several instances that any considerable irritation of a specimen during the swallowing process was almost invariably followed by a reversion and ejection of the food. A worm three fourths swallowed would be ejected by a sort of antiperistalsis, which was more rapid than the swallowing had been.

From what has just been stated it need hardly be observed that attempts to feed specimens with bits of blotting paper, or other such materials, were uniformly negative in character.

The feeding experiments with other species were too limited to justify any special attention in this connection. In most cases no difficulty was encountered in inducing species of *Sagartia* to take food of almost any sort.

Burrowing Reaction. — Attention has been directed in an earlier connection to the fact that considerable variability is evident among various specimens as to the matter of burrowing, or tube-building. It may not be without some interest to briefly cite a few details along this line. It is one of the curious features in the activities of *Eloactis* that among a dozen specimens put into an aquarium the most remarkable difference of behavior in this respect may be seen. Most will show early signs of activity, and soon bury their bodies as completely as possible, and assume an erect position. Others appear to go through the efforts but in a most futile way. Left over night the aquarium will show in the tracks over the surface of the sand the varied movements made in this way. Still other specimens seem to show no effort whatever to burrow, but lie indifferently upon the surface, hardly showing signs of life except as they are stimulated by some means. This may continue somewhat indefinitely. But after a time a change may come over one of these sluggish specimens and it sets about constructing a burrow all at once, as it were, and within a night will have taken up the characteristic attitude of its kind. If now it be dug out and left again upon the sand it may promptly readjust itself again in a burrow, or it may remain for some days in the same indifferent aspect. Specimens which first bury themselves are usually prompt to build fresh burrows if dug out of the earlier ones.

The facts herein portrayed suggest several interesting inferences and inquiries by way of conclusion.

1. It seems clear that in the behavior of actinians toward light one is forced to recognize that certain species have sensory perceptions of photic stimuli quite as well defined as exist in such organisms as the earthworm, clam, etc. And while in this group of cœlenterates no such definite sensory organs are known as those found in many medusæ, the Hertwigs have described certain ectodermal cells which they have designated as sensory in function. It is not without some warrant that we may conclude that the various aspects of behavior under consideration are more or less definitely correlated with sensory structures and perhaps nerve cells.

2. Loeb, who has studied certain aspects of the behavior of *Cerianthus membranaceus* (" Physiology of the Brain," pp. 56–59), attributes them to the influence of two tropic forces, namely, geotropism and heliotropism. " Positive geotropism and positive stereotropism cause the Cerianthi to burrow in the sand vertically, and positive geotropism keeps them permanently in the burrow."

I have elsewhere shown the inadequacy of this explanation as applied to tube-dwelling annelids. I believe the facts under review may likewise be better understood and more consistently explained by other modes. Certainly the factor of light must be reckoned with as potent in the behavior of the several species studied. Again the variable behavior of these creatures in their burrowing habits is not easily accounted for on the usual theory of tropisms. Furthermore, it seems highly probable that in some cases the food-taking habit may sustain a relation to the general tube-dwelling habit.

3. Finally, as one considers the interesting facts as to the distribution of these light-reacting anemones the foregoing inferences are strongly corroborated. It is not necessary to review these facts in detail. It will be recalled that the observations of Quatrefages and Haime, already cited, had to do with species of *Cerianthus* and *Edwardsia* both of burrowing habit. Those of the Hertwigs were made on a species of *Cladactis*, an inhabitant of the deep sea. The observations of Jourdan were made on a species of *Paractis*, whose habit is not given, though species of this genus taken by the Challenger Expedition were also from the deep sea.

Of the species which have come under my own observations as light-perceptive, two are tube-dwelling, and one free-living, but secreting itself under various forms of cover, or occasionally burrowing in sand. Certainly neither geotropism nor stereotropism are equally or reasonably applicable as explanations of all these varied conditions and habits. I believe we are therefore forced to the conclusion that physiological conditions of adaptation are primarily involved, and that the various phases of behavior are so many expressions of such adjustments.

Syracuse University,
January 1, 1907.

ON THE ANATOMY OF THE CENTRAL NERVOUS SYSTEM OF THE NINE-BANDED ARMADILLO (TATU NOVEMCINCTUM LINN.).[1]

LILY C. SHUDDEMAGEN.

The present paper is a contribution to the macroscopical anatomy of the brain, spinal cord, cranial and spinal nerves of the nine-banded armadillo.

There appears to be no literature on any portion of the central nervous system except the brain in any edentate, with the exception of Pouchet's classic account of *Myrmecophaga ;* with this omission the relations of the spinal cord in this group are unknown, and the present description of that organ complex is an attempt to fill this hiatus in our knowledge. A list of the memoirs treating of the anatomy of the brain is appended at the end of the present paper ; of those memoirs, the ones by Gervais (1869), Pouchet (1869), and notably Smith (1899) are the most important. Smith is the only writer who mentions the brain of the particular species examined by me, but gives no figures of it ; and indeed, our knowledge of the general anatomy of this species is much more scant than of various other armadillos,— even the rare *Chlamydophorus.*

Comparisons of the brain of this species are made with the brains of other described Dasypodidæ, and I have followed Smith's nomenclature of the parts.

The material used consisted of four specimens, two males and two females, procured in the neighborhood of Austin, Travis County, Texas. Two of these were preserved in formalin and two in alcohol.

This work has been done entirely under the direction of Prof. Thos. H. Montgomery, Jr., and the writer is under great obligation to him for his helpful suggestions, and kindly sympathy and constant encouragement during the preparation of this memoir.

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[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 75.

1. The Brain.

General Topography.

The brain is almost twice as long as broad. The bulbus olfactorius forms the most anterior, and the medulla oblongata its most posterior parts. The cerebrum broadens out posteriorly, and, on lateral view, is not quite as high as the cerebellum. The general shape is much like that of the lower mammals.

(*a*) *Prosencephalon.* — The prosencephalon is composed of the following parts: bulbus olfactorius, tuberculum olfactorium, lobus pyriformis, pedunculus olfactorius, locus perforatus, and the cerebral hemispheres.

The *bulbus olfactorius* (Pl. XXIV., Fig. 1, *Bul. Olf.*) is the most anterior part of the brain, and is relatively enormous. From a ventral view, it is seen to be heartshaped with the apex pointing forward. The ventral surface is indented by almost parallel furrows or sulci, running at right angles to the long axis of the brain. The dorsal surface (Fig. 4) is spherically rounded and smooth. It is placed somewhat ventral to the cerebral hemispheres (Fig. 2), so that over half of its dorsal surface is overlapped by them. From the anterior part of the bulbus olfactorius the olfactory nerve spreads out in a great fan-shaped mass.

The *tuberculum olfactorium* (Pl. XXIV., Fig. 1, *Tub. Olf.*) is a large oval area slightly raised above the surrounding regions. It is separated from the bulbus olfactorius by the pedunculus olfactorius. Its surface is not smooth, but somewhat tuberculated. It reaches a relatively large size in the armadillo.

The *lobus pyriformis* is visible along the lateral surface of the brain, just posterior to the tuberculum olfactorium; it consists of an anterior lobe (*Lob. Pyr. A.*, Fig. 2), and a posterior (*Lob. Pyr. P.*, Fig. 1).

The *pedunculus olfactorius* (Pl. XXIV., Fig. 3, *Ped. Olf.*) is to be seen only in a lateral view of the brain. It connects the bulbus olfactorius with the remainder of that organ. In both dorsal (Pl. XXIV., Fig. 4), and ventral (Fig. 1), views it is hidden by the cerebral hemispheres and the oblique position of the bulbus olfactorius.

The *locus perforatus* (Pl. XXIV., Fig. 1, *Loc. Perf.*) is the depressed, quadrilateral area immediately anterior to the optic chiasma.

The *cerebral hemispheres* (Pl. XXIV., Figs. 2 and 4) show as high a development as any of the armadillos figured by Smith. The short *anterior rhinal fissure* (Pl. XXIV., Fig. 4, *Fis. Rh. A.*) begins in the boundary between the bulbus olfactorius and the hemispheres. It extends obliquely upwards for about a fourth of the length of the hemispheres. The *posterior rhinal fissure* (Pl. XXIV., Fig. 4, *Fis. Rh. P.*) begins near the posterior border of the hemispheres and runs horizontally towards the anterior part of the hemispheres, where it joins the sulcus β (Pl. XXIV., Fig. 4, β). In the most dorsal part of the hemispheres, the sulci γ and δ (Pl. XXIV., Fig. 4) are faintly developed. The latter of these two sulci corresponds to the suprasylvian sulcus of other mammals. On the mesial surface of the two hemispheres the sulcus limitans pallii (Pl. XXIV., Fig. 3, *Sul. L.*) is found.

In this animal, as in all mammals, a series of nerve fibers, or *commissures*, serve to connect homologous areas of the two hemispheres.

The most dorsally placed commissure is in the form of an inverted, obliquely placed U (Pl. XXIV., Fig. 3, *Cor. Cal.*). The arms of the U are formed by the corpus callosum (Pl. XXIV., Fig. 3, *Cor. Cal.*), and the ventral and dorsal psalterium (Pl. XXIV., Fig. 3, *Psal. V.* and *D.*). The curve of the U is formed by the splenium (Pl. XXIV., Fig. 3, *Spl.*). This commissure is placed more nearly vertical, and is rather smaller than in most of the edentate brains figured by Smith. There is really no apparent distinction between the dorsal and ventral psalterium. The psalterium is slightly longer than the corpus callosum. The two arms of this dorsal commissure are in contact with each other for the greater part of their extent, only the most ventral part of the psalterium extends a little further ventrally than the corpus callosum. The interval between the two arms of the dorsal commissure is called the septum lucidum in human anatomy. In the edentates, Smith calls this the paracommissural body. But since the two arms of the commissure are in contact with each other for the greater part of their extent, there is practically no septum lucidum or paracommissural body, in this armadillo.

The *anterior commissure* (Pl. XXIV., Fig. 3, *Com. A.*) is of fairly large size. It is a rather cylindrical bundle of fibers and connects the pyriform lobes. Because of the relatively large size of the pyriform lobes, the anterior commissure attains its increase of size. In the armadillos, all the parts of the brain connected with the sense of smell, reach relatively large dimensions.

(*b*) *Thalamencephalon.* — The thalamencephalon is the second embryological division of the brain, and consists of that part which bears the optic thalami, the infundibulum, pituitary body, and pineal body.

The *optic thalami* (Pl. XXV., Fig. 7, *Opt. Th.*) and the corpora quadrigemina (Pl. XXV., Fig. 7, *Cor. Q.*) form a large area of quadrilateral shape. The optic thalami are separated from each other, in the median line, by the third ventricle. They are connected across this ventricle by means of the commissura molli. This extends across the slit-like third ventricle as a large cylindrical mass of fibers (Pl. XXIV., Fig. 3, *Com. Mol.*). Thus the third ventricle becomes reduced to a narrow circular channel surrounding the commissura molli.

The floor of the third ventricle is drawn downward into a funnel-shaped pouch, the *infundibulum* (Pl. XXIV., Fig. 3, *Inf.*).

The *hypophysis* (Pl. XXIV., Fig. 3, *Hyp.*) is attached to the ventral part of the infundibulum.

The *pineal body* (Pl. XXV., Fig. 7, *Cor. Pin.*) lies in a shallow groove of the anterior corpora quadrigemina, just posterior to the third ventricle.

The *third ventricle* (Pl. XXV., Fig. 7, *Ven. III*) opens into the two first ventricles (Pl. XXV., Fig. 7, *Ven. I*) by means of the foramen of Monroe (Pl. XXV., Fig. 7, *For. M.*). Out of the posterior part of the third ventricle, the aqueduct of Sylvius (Pl. XXIV., Fig. 3, *Aq. Syl.*) opens and passes into the fourth ventricle.

The *II, or optic nerve* (Pl. XXIV., Fig. 1, *II*) comes off from the ventral surface of the brain, just a little anterior to the infundibulum. It is of very small size, because of the great diminution of the visual acuteness and consequent reduction of the size of the eye.

The *IV, or pathetic nerve* (Pl. XXIV., Fig. 1, *IV*) arises from the ventral surface of the brain, just posterior to the infundibulum.

(*c*) *Mesencephalon.* — The mesencephalon is that embryological division of the brain which gives rise to the corpora quadrigemina and the crura cerebri.

The *corpora quadrigemina* (Pl. XXV., Fig. 7, *Cor. Q.*) lie immediately posterior to the optic thalami. The anterior pair of the corpora quadrigemina forms an area slightly elevated above the level of the optic thalami. Just posterior to them, the posterior pair of the corpora quadrigemina rise to a much higher level (Pl. XXIV., Fig. 3, *Cor. Q.*) ; their most dorsal point comes up almost to the level of the cerebral hemispheres. The corpora quadrigemina are wedged between the cerebellum and the cerebral hemispheres. In the armadillo, they are not separated across the middle, but form one body in which separation is only faintly indicated by a shallow longitudinal furrow. The reduction in the size of the anterior pair of the corpora quadrigemina is probably due to the waning importance of the sense of sight. The posterior corpora quadrigemina retain their large size, or perhaps even show an increase in size, because they are not connected as directly with the sense of sight.

The *crus cerebri* arises from under the optic tract as a faint, indistinct band of fibers, runs backwards and disappears under the pons Varolii.

(*d*) *Metencephalon.* — The embryological division of metencephalon gives rise, in the adult, to the cerebellum.

Viewed dorsally (Pl. XXIV., Fig. 4), the cerebellum presents a somewhat triangular shape, where the paraflocculi (Pl. XXIV., Fig. 4, *Par. Fl.*) and the posterior lobe (Pl. XXIV., Fig. 4, *Lob. P.*) form the three angles. The cerebellum is much convoluted, as is the case in all mammals. Its greatest diameter is transverse. This large cerebellar mass hides from view the entire fourth ventricle except the most posterior part (Pl. XXIV., Fig. 4, *Ven. IV*). The cerebellum is supported and connected with the brain stem by two cerebellar peduncles (Pl. XXV., Fig. 7, *Ped. Cer.*). Anteriorly, the cerebellum is closely adapted to the contour of the cerebral hemispheres. It projects forward sufficiently to hide the posterior corpora quadrigemina completely.

The most lateral projections of the cerebellum are two fairly large sized bodies. These bodies, composed of a number of folia and separated almost entirely from the remainder of the cerebellum by a fissure, are the *lobi flocculi.*

Each of these *lobi flocculi* consists of two distinct parts, the flocculus (Pl. XXIV., Figs. 1 and 2, *Floc.*) and the paraflocculus (Pl. XXIV., Figs. 2 and 4, *Par. Fl.*). The latter is much the largest of the two, and almost completely hides the former from view. From a dorsal view the paraflocculus (Pl. XXIV., Fig. 4, *Par. Fl.*) appears as a crescentic mass of folia, forming the lateral projections of the cerebellum.

Aside from the *lobi flocculi,* the remainder of the cerebellum may be divided into three lobes, the lobus anticus (Pl. XXIV., Fig. 4, *Lob. A.*), the lobus centralis (Pl. XXIV., Fig. 4, *Lob. C.*), and the lobus posticus (Pl. XXIV., Fig. 4, *Lob. P.*).

The *lobus anticus* (Pl. XXIV., Figs. 2, 3, and 4, *Lob. A.*) is separated from the posterior part of the cerebellum by the fissura prima (Pl. XXIV., Figs. 2, 3, and 4, *Fis. 1*). It is clearly visible in a dorsal view of the brain (Pl. XXIV., Fig. 4, *Lob. A.*), and is not hidden between the lobus centralis and the cerebral hemispheres, as is the case in the *Chlamydophorus* (Smith, 1899, Fig. 34) or in *Xenurus* (Smith).

The *lobus centralis* (Pl. XXIV., Figs. 2, 3 and 4, *Lob. C.*) is separated from the lobus posticus by means of the fissura secunda (Pl. XXIV., Figs. 2, 3 and 4, *Fis. 2*). It constitutes the largest and most complex part of the cerebellum. It is a large irregular area which has bulged forward and laterally, wedging its way between the lobus anticus and the lobus flocculus.

The *lobus posticus* (Pl. XXIV., Figs. 2, 3 and 4, *Lob. P.*) is the most caudal part of the cerebellum. It is small, consisting of but few folia, and covers over almost completely the posterior part of the fourth ventricle.

(*e*) *Myelencephalon.* — The embryonic division of myelencephalon gives rise, in tne adult, to the medulla oblongata and the pons Varolii.

The *medulla oblongata* (Pl. XXIV., Fig. 4, *Med. Obl.*) is the most posterior part of the brain, and is continued directly into the spinal cord. In the medulla oblongata is the fourth ventricle, roofed over by a thin membrane. The greatest part of the medulla oblongata is covered over by the cerebellum.

The *pons Varolii* (Pl. XXIV., Fig. 3, *Pons*) forms the most anterior part of the hind brain. It is a pair of slight elevations on the ventral surface of the brain, a little posterior to the infundibulum.

From the medulla oblongata arise all the remainder of the cranial nerves, from the V to the XII inclusive.

The *V, or trigeminal nerve* (Pl. XXIV., Fig. 1, *V*) arises from the pons Varolii. It soon divides into two branches, the most lateral of which subdivides again.

The *VI, or abducent nerve* (Pl. XXIV., Fig. 1, *VI*) arises in the region of the pons Varolii, and runs to the external rectus eye muscle.

The *VII, or facial nerve* (Pl. XXIV., Fig. 1, *VII*) arises in close connection with the VIII nerve, in the region just laterad of the pons Varolii. It soon subdivides into branches.

The *VIII, or auditory nerve* (Pl. XXIV., Fig. 1, *VIII*) arises with the VII nerve from the same part of the brain. It runs directly outwards and enters the cochlea of the ear.

The *IX, or glosso-pharyngeal nerve* (Pl. XXIV., Fig. 1, *IX*) arises by several roots, from the ventral surface of the medulla.

The *X, or pneumogastric nerve* (Pl. XXIV., Fig. 1, *X*) arises by several roots from the medulla, just posterior to the IX nerve.

The *XI, or spinal accessory nerve* (Pl. XXIV., Fig. 1, *XI*) arises by several roots from the ventral surface of the medulla and the spinal cord. Some of its roots arise from the spinal cord, as far back as the fourth cervical nerve.

The *XII, or hypoglossal nerve* (Pl. XXIV., Fig. 1, *XII*) arises by several roots from the medulla oblongata, just posterior to the origin of the XI nerve.

2. Spinal Cord.

The spinal cord is cylindrical, but somewhat flattened dorsoventrally. In the cervical and sacral regions, it has a slight enlargement from which the nerves of the brachial and lumbosacral plexuses are given off. In the sacral region the cord breaks up into a number of fine nerves which occupy the vertebral canal as the cauda equina. These nerve branches pass out, pair by pair, from between the caudal vertebræ and supply the muscles of the tail.

The most anterior division of the spinal nerves is the cervical (Pl. XXVI., *C. 1–C. 8*). Of these there are eight pairs.

Of the thoracic nerves (Pl. XXVI., *T. 1–T. 10*) there are ten pairs.

The lumbar region is very short, containing six pairs of nerves (Pl. XXVI., *L. 1–L. 6*).

The sacral nerves (Pl. XXVI., *S. 1–S. 8*) are eight in number.

The exact number of the caudal nerves was not ascertained by me. But they are quite numerous, possibly as many as fifteen to twenty pairs.

(*a*) *Cervical Plexus.* — The cervical plexus (Pl. XXVI., *C. 1–C. 8*) is composed of the dorsal branches of the eight pairs of cervical nerves. These branches pass almost vertically upwards, interlace, and supply the dorsal neck muscles. On Pl. XXVI., on the right hand side of the drawing, are shown the dorsal branches of the cervical nerves.

(*b*) *Brachial Plexus.* — The brachial plexus is composed of the large ventral branches of the third, fourth, fifth, sixth, seventh, and eighth cervical nerves, and the first and second thoracic nerves. The formation of the plexus is due to the union of the several nerves, by means of strong connecting branches. The plexus lies in the axilla, and all the component nerves pass out laterally, almost parallel to the first rib. By means of its branches, the arm and shoulder are innervated.

The three *subscapular nerves*, the cranial (Pl. XXVI., *Sub. Sc. 1*), the middle (Pl. XXVI., *Sub. Sc. 2*), and the caudal (Pl. XXVI., *Sub. Sc. 3*), all supply muscles on the ventral surface of the scapula. The cranial subscapular nerve (Pl. XXVI., *Sub. Sc. 1*) arises from the third, fourth, and fifth cervical nerves. The middle subscapular nerve (Pl. XXVI., *Sub. Sc. 2*) arises from the sixth cervical nerve. The caudal subscapular nerve (Pl. XXVI., *Sub. Sc. 3*) arises from the sixth, seventh, and eighth cervical nerves.

The *suprascapular nerve* (Pl. XXVI., *Sup. Sc.*) arises from the fifth cervical nerve. It passes onto the dorsal side of the scapula and enervates the supraspinatus and infraspinatus muscles.

The *axillary nerve* (Pl. XXVI., *Ax.*) arises from the fifth and sixth cervical nerves. It supplies some muscles in the upper arm.

The *radiales nerve* (Pl. XXVI., *Rad.*) is one of the three nerves that supply the lower arm and hand. It arises from the sixth, seventh, and eighth cervical nerves.

The *medianus nerve* (Pl. XXVI., *Med.*) also principally supplies the muscles of the forearm and hand. It arises from the seventh, and eighth cervical, and the first thoracic nerves.

The *ulnaris nerve* (Pl. XXVI., *Uln.*) is the third lower arm and hand nerve. It arises from the eighth cervical, and first and second thoracic nerves.

(*c*) *Thoracic Plexus.*— From the first, second, and third thoracic nerves arise three ventral branches which pass out laterally and unite into a little separate plexus (Pl. XXVI., *X.*) Then this plexus gives off three main branches which subdivide again and again. All of these branches supply the great lateral skin muscle which is attached along the whole length of the armor. A plexus like this, to my knowledge, is not present in any other mammal. It has probably arisen because of the great development of the large skin muscle, which attaches to the sides of the armor and functions in drawing the animal together in a ball. Because of its origin from the thoracic nerves, I have taken the liberty of naming it the thoracic plexus.

The remainder of the thoracic nerves are arranged similarly to those of other mammals. They divide into two branches almost immediately after leaving the intervertebral foramina. The dorsal branches supply the superficial muscles of the back, while the ventral branches run along the ribs as the intercostal nerves.

(*d*) *Lumbar Nerves.* — The first three lumbar nerves take no part in the formation of the lumbo-sacral plexus. The ventral branch of the first lumbar nerve divides into two branches, the ilio-hypogastric (Pl. XXVI., *Il. Hyp.*), and the ilio-inguinal (Pl. XXVI., *Il. Ing.*). The ventral branch of the second lumbar nerve forms the genito-crural nerve (Pl. XXVI., *Gen. Cr.*). The third lumbar nerve forms the external cutaneous nerve (Pl. XXVI., *Ext. Cut.*).

(*e*) *Lumbo-Sacral Plexus.* — The lumbo-sacral plexus is composed of the fourth, fifth, and sixth lumbar, and the eight sacral nerves. These nerves are all interconnected by strong branches, and they supply the muscles of the thigh and lower limb.

The *anterior crural nerve* (Pl. XXVI., *Ant. Cr.*) is composed of parts of the fourth, fifth, and sixth lumbar nerves. It supplies some of the upper thigh muscles.

The *obturator nerve* (Pl. XXVI., *Obt.*) arises from the sixth lumbar and first sacral nerves. It also goes to supply some of the upper thigh muscles.

The *sciatic major nerve* (Pl. XXVI., *Sc. Maj.*) arises from the sixth lumbar, and first, second, and third sacral nerves. This is the great nerve of the posterior limb. It soon divides into the tibialis (Pl. XXVI., *Tib.*), the perroneus (Pl. XXVI., *Per.*), the gluteous (Pl. XXVI., *Glut.*), and the sciatic minor nerve (Pl. XXVI., *Sc. Min.*).

The *pudendus nerve* (Pl. XXVI., *Pud.*) arises from the fourth sacral nerve.

The *cutaneous femoris nerve* (Pl. XXVI., *Cut.*) arises from the fifth sacral nerve.

General Remarks.

The brain has been previously described for the following Dasypodids :

The brain of *Chlamydophorus truncatus* has been figured and described by Smith (1899) and Pouchet (1869). Hyrtl (1855) gives just a few brief notes on the brain, without any figures.

Dasypus sexcinctus has been figured and described by Smith (1899), Turner (1867), and Pouchet (1869).

Priodon gigas has been figured by Pouchet (1868 and 1869), and mentioned by Smith (1899).

Tolypentes tricinctus has been mentioned by Smith (1899), and figured and described by Gervais (1869).

Tatu novemcinctum has been mentioned by Smith (1899), without figures.

Tatu peba has been figured and described by Smith (1899) and Rapp (1852).

Xenurus unicinctus has been figured and mentioned by Smith (1899) and Garrod (1878).

Dasypus villosus has been figured and described by Smith (1899).

Smith's (1899) work on the Armadillos is by far the most important, and for this reason I have compared the species under present consideration with his descriptions.

The brain of *Tatu novemcinctum* shows less similarity with the genus *Chlamydophorus*, than with the brain of any other genus of armadillo. To judge by the figure of *Xenurus unicinctus* given by Garrod (1878), there seems to be greater similarity of the brain of *Tatu* with *Xenurus* than with any other genus of armadillo. The fissures, sulci, and the general shape and contour of these two brains have very many points in common. However, much more detailed study must be made of all the species of *Armadillo* before one could venture to assert this with any degree of certainty.

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DESCRIPTION OF THE PLATES.

The following abbreviations have been used :

(Greek letters denote sulci.)

Ant. Cr.	anterior crural.
Aq. Syl.	aqueductus Sylvii.
Ax.	axillary.
Bul. Olf.	bulbus olfactorius.
C. 1–8.	cervical nerves.
Com. A.	commissura anterior.
Com. Mol.	commissura mollis.
Com. P.	commissura posterior.
Cor. Cal.	corpus callosum.
Cor. Pin.	corpus pineale.
Cor. Q.	corpora quadrigemina.
Cut. Fem.	cutaneus femoris posterior.
Ex. Cut.	external cutaneous.
Fis. 1.	fissura prima.
Fis. 2.	fissura secunda.

Fis. Rh. A.	anterior rhinal fissure.
Fis. Rh. P.	posterior rhinal fissure.
Floc.	flocculus.
For. M.	foramen of Monro.
Gen. Cr.	genito-crural.
Glut.	glutæus inferior.
Hipp.	hippocampus.
Hyp.	hypophysis.
Il. Hyp.	ilio-hypogastric.
Il. Ing.	ilio-inguinal.
Inf.	infundibulum.
L. 1–10.	lumbar nerves.
Lob. A.	lobus anticus.
Lob. C.	lobus centralis.
Lob. P.	lobus posticus.
Lob. Pyr. A.	lobus pyriformis anterior.
Lob. Pyr. P.	lobus pyriformis posterior.
Loc. Perf.	locus perforatus.
Med.	medianus.
Med. Obl.	medulla oblongata.
Obt.	obturator.
Opt. Th.	optic thalami.
Par. Fl.	paraflocculus.
Ped. Cer.	pedunculi cerebelli.
Ped. Olf.	pedunculus olfactorius.
Per.	peroneus.
Psal. D.	psalterium dorsale.
Psal. V.	psalterium ventrale.
Pud.	pudendus.
Rad.	radialis.
S. 1–8.	sacral nerves.
Sc. Maj.	major sciatic.
Sc. Min.	minor sciatic.
Spl.	splenium.
Sub. Sc. 1.	cranial subscapularis.
Sub. Sc. 2.	middle subscapularis.
Sub. Sc. 3.	caudal subscapularis.
Sul. L.	sulcus limitans pallii.
Sup. Sc.	suprascapularis.
T. 1–10.	thoracic nerves.
Tib.	tibialis.
Tr. Opt.	tractus opticus.
Tub. Ac. L.	tuberculum acusticum laterale.
Tub. Ac. M.	tuberculum acusticum median.
Tub. Olf.	tuberculum olfactorium.
Uln.	ulnaris.
Ven. I.–IV.	ventricles I.–IV.
I.–XII.	cranial nerves.

EXPLANATION OF PLATES.

All the figures are from enlarged freehand sketches. The figures on Plates I. and II. were drawn twice natural size, and then reduced about one third in the reproduction. Plate III. was drawn natural size, and then reduced about one half in the reproduction.

PLATE XXIV.

FIG. 1. Ventral view of the brain.

FIG. 2. Lateral view.

FIG. 3. Median longitudinal section.

FIG. 4. Dorsal view.

FIG. 5. Ventral view of a late fœtal brain ; length of the fœtus was about 15 cm.

FIG. 6. Dorsal view of the same late fœtal brain as Fig. 5.

Fig. 1

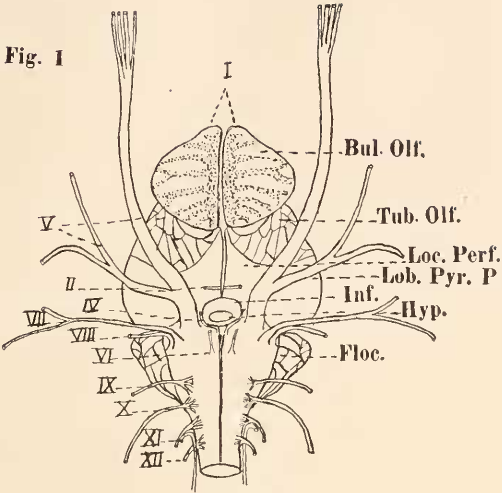


Fig. 6

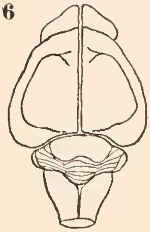


Fig. 5

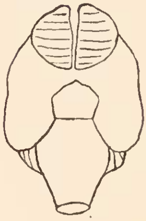


Fig. 2

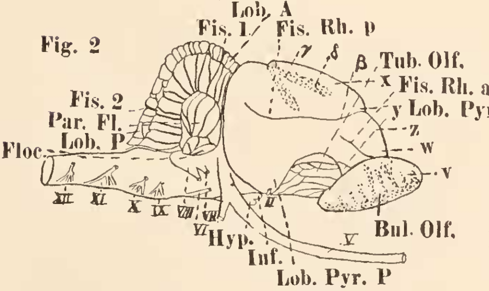


Fig. 4

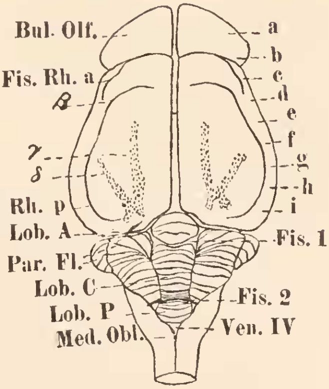


Fig. 3

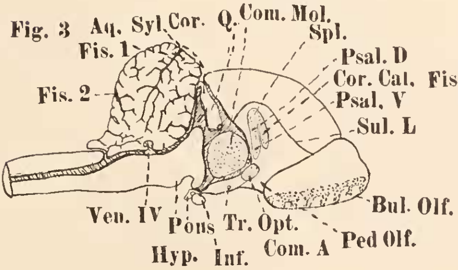


PLATE XXV.

FIGS. 7–11. Horizontal, longitudinal sections of the brain. The dotted parts of the figures show the ventricles.

FIG. 7. The dorsal surface of a horizontal section; taken in plane *v* of Fig. 2.

FIG. 8. The dorsal surface of a horizontal section, taken in the plane *w* of Fig. 2.

FIG. 9. The dorsal surface of a horizontal section, taken in the plane *z* of Fig. 2.

FIG. 10. The dorsal surface of a horizontal section, taken in the plane *y* of Fig. 2.

FIG. 11. The dorsal surface of a horizontal section, taken in plane *x* of Fig. 2.

FIGS. 12–20 are cross-sections of the brain, beginning at the anterior end.

FIG. 12. The caudal surface of a cross-section taken in the plane *a* of Fig. 4.

FIG. 13. The caudal surface of a cross-section taken in the plane *b* of Fig. 4.

FIG. 14. The caudal surface of a cross-section taken in the plane *c* of Fig. 4.

FIG. 15. The caudal surface of a cross-section taken in the plane *d* of Fig. 4.

FIG. 16. The caudal surface of a cross-section taken in the plane *e* of Fig. 4.

FIG. 17. The caudal surface of a cross-section taken in the plane *f* of Fig. 4.

FIG. 18. The caudal surface of a cross-section taken in the plane *g* of Fig. 4.

FIG. 19. The caudal surface of a cross-section taken in the plane *h* of Fig. 4.

FIG. 20. The caudal surface of a cross section taken in the plane *i* of Fig. 4.

Fig. 7

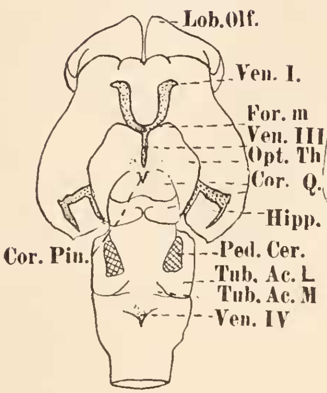


Fig. 8

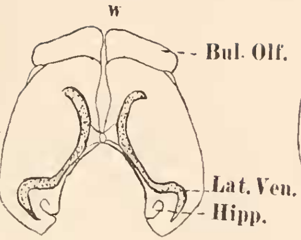


Fig. 9

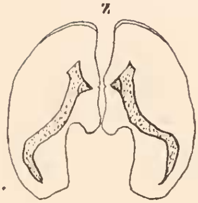


Fig. 10

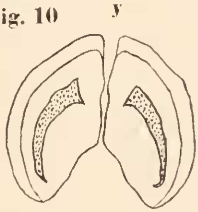


Fig. 11

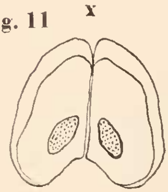


Fig. 12

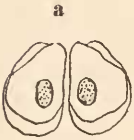


Fig. 13

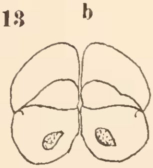


Fig. 14

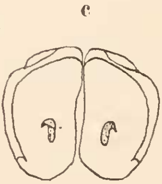


Fig. 15

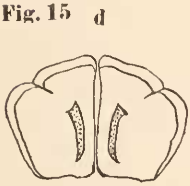


Fig. 16

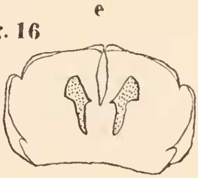


Fig 17

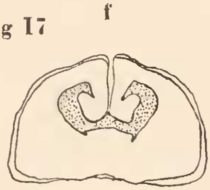


Fig. 18

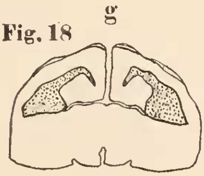


Fig. 19

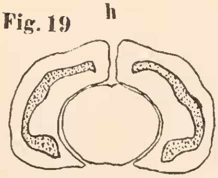


Fig. 20

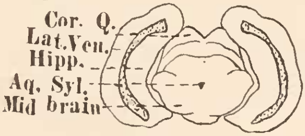
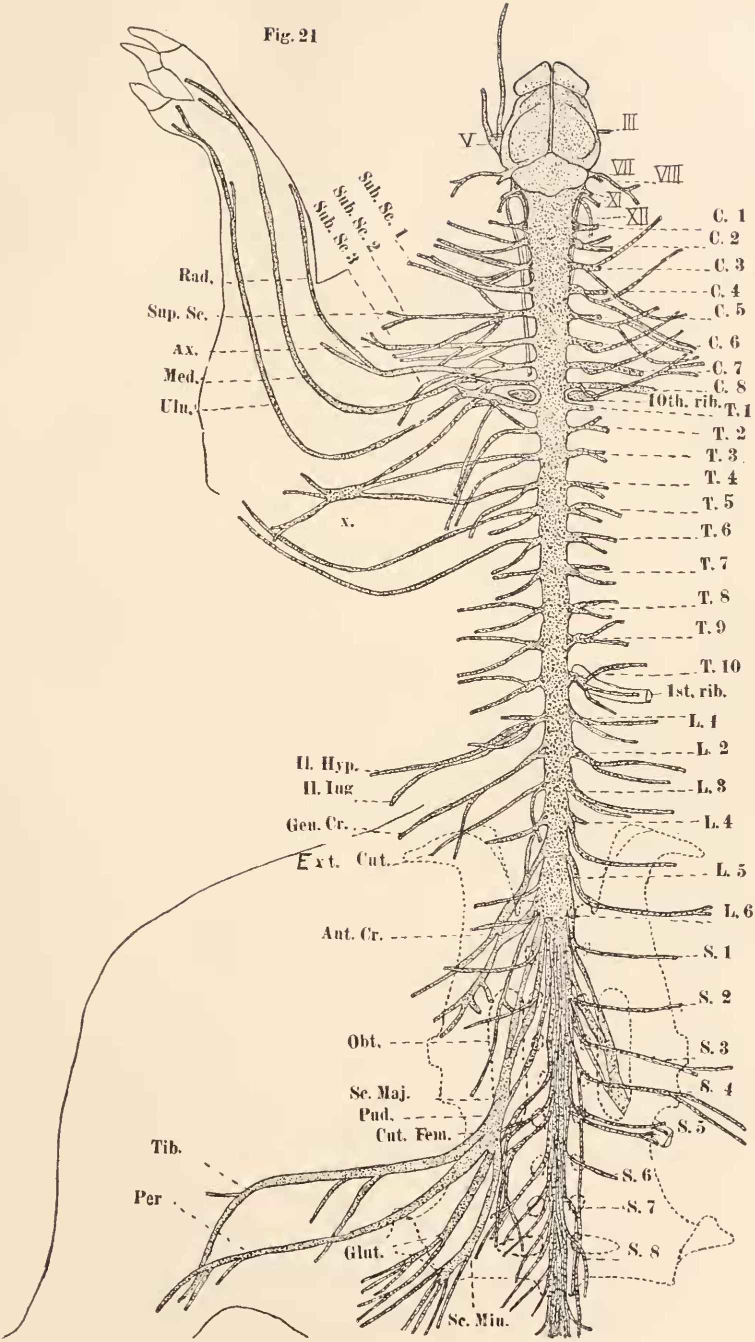


PLATE XXVI.

FIG. 21. A drawing of the entire central nervous system. The dotted lines show the outline of the sacrum in its natural relation to the spinal nerves. The nerves on the left hand side of the figure are all the ventral branches of the spinal nerves. On the right hand side of the figure, the more superficial branches of the spinal nerves are shown.

Fig. 21


V
III
VII
VIII
XI
XII
Sub. Sc. 1
Sub. Sc. 2
Sub. Sc. 3
Rad.
Sup. Sc.
Ax.
Med.
Ulu.
x.
C. 1
C. 2
C. 3
C. 4
C. 5
C. 6
C. 7
C. 8
10th. rib.
T. 1
T. 2
T. 3
T. 4
T. 5
T. 6
T. 7
T. 8
T. 9
T. 10
1st. rib.
L. 1
L. 2
L. 3
L. 4
L. 5
L. 6
Il. Hyp.
Il. Ing.
Gen. Cr.
Ext. Cut.
Ant. Cr.
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Sc. Maj.
Pud.
Cut. Fem.
Tib.
Per
Glut.
Sc. Min.
S. 1
S. 2
S. 3
S. 4
S. 5
S. 6
S. 7
S. 8

Vol. XII. *April, 1907.* *No. 5.*

BIOLOGICAL BULLETIN

NOTE ON THE CHROMOSOME-GROUPS OF METAPODIUS AND BANASA.[1]

EDMUND B. WILSON.

I am led to publish the following preliminary note lest confusion should arise from the peculiar relations of the chromosomes in *Banasa calva* described in the second of my "Studies on Chromosomes,"[2] which have now, I believe, become explicable as a result of additional studies on *Banasa*, but more especially on the genus *Metapodius*. At the time these relations were described in *Banasa calva* they appeared to be unique in that both an unpaired chromosome (apparently an "accessory" chromosome) and a typical pair of unequal idiochromosomes are present in the same species, and four classes of spermatozoa are accordingly formed. The coexistence of these two forms of chromosomes in the same individual has already been considered by more than one writer as a serious difficulty in the way of my general interpretation of the significance of these chromosomes in sex-production. It was (and is) my view that the "accessory" chromosome is the homologue of the large idiochromosome and like the latter is distinctive of the female-producing spermatozoa. It seemed, no doubt, an obstacle to this view that an unpaired or heterotropic chromosome should coexist with a pair of idiochromosomes in the same species, and that it should in one class of spermatozoa be associated with a large idiochromosome, in another class with a small one. With the material at my command (which included only two testes from the Paulmier collection) I was not in a position to

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[1] The material on which the observations were made is part of a series procured in the course of an extended collecting trip to the south and west in the summer of 1906, the cost of which was in part defrayed by a grant from the Carnegie Institution of Washington. The results will be published in a more extended form hereafter.

[2] *Journ. Exp. Zool.* II., 4, 1905.

meet this difficulty or to give an adequate explanation of the facts; and for a time, I even suspected that the material might be pathological. Recently, however, I have found a similar, though not quite identical, condition in a species of *Metapodius*, and have been able to study the facts more thoroughly. In this form, too, an unpaired chromosome coexists with a typical pair of idiochromosomes (and a pair of *m*-chromosomes as well); but the facts clearly show that it is not of the same nature as the "accessory" or "heterotropic" chromosome of the usual type, and is without constant relation to sex-production. The idiochromosomes show the usual relation, the large one passing to the female producing pole and the small one to the male-producing pole. A comparison of different individuals shows beyond doubt that the unpaired chromosome may be either present or absent in either the male or female, and hence is without significance in sex-production. It is in fact a kind of supernumerary chromosome, which I shall designate as the "*s*-chromosome" in order to distinguish it from the odd sex-chromosome of the usual type — variously known as the "accessory chromosome" (McClung), "heterotropic chromosome" (Wilson), or "monosome" (Montgomery).

1. METAPODIUS TERMINALIS Dall.[1]

The present account will give only the facts that bear directly on the case of *Banasa calva*. The genus *Metapodius* is, I believe,

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[1] The following description will be found to differ widely from that given for the same species by Montgomery (*Trans. Am. Phil. Soc.*, N. S., XXI., 3, 1906), who states that there are 21 spermatogonial chromosomes and an ordinary large odd chromosome in the second division. Professor Montgomery has kindly sent me some of his own material, collected in Pennsylvania, a study of which has convinced me of the correctness of his account. My own material is from New Jersey, North Carolina, South Carolina, Georgia and Ohio; and there can be no doubt of the identification since every original specimen is in my possession (as is the case with all my new material). Through the courtesy of Dr. Uhler I have been enabled to compare these specimens with those in his collection (with which they exactly agree); and they have also been examined by several competent hemipterists, including Mr. Otto Heidemann, of Washington, and Mr. H. G. Barber, of New York, and pronounced by them to be typical *terminalis*. As will be shown, different individuals among these specimens show constant and characteristic differences in the chromosome-groups; but none show less than 22 chromosomes, and none possess a large odd chromosome. The same is true of *M. femoratus* Fab., and *M. granulosus* Dall., both of which, like *terminalis*, possess a typical pair of idiochromosomes. This contradic-

in a somewhat plastic condition as regards the chromosomes, and presents certain variations in the number of the larger chromosomes that need not here be described, since they do not affect the relations to be considered. Alone among all the Coreidæ thus far examined, the three species of *Metapodius* possess a typical pair of idiochromosomes along with a typical pair of *m*-chromosomes — a fact which proves the validity of the distinction between these two forms of chromosomes drawn in my second study. The idiochromosomes are distinctly, though not greatly, unequal in size ; and as usual among the Hemiptera, they remain separate as univalents in the first maturation division, but conjugate at the end of this division to form an unequal bivalent. In the greater number of individuals (which may be classed together as "Type A") the first division shows 13 chromosomes (Fig. 1, *b*) and the second 12. In the most usual arrangement the two idiochromosomes (*I* and *i*) lie in the first division not far apart, outside an irregular ring formed of nine larger bivalents, in the position typical of the odd chromosome in other coreids. Near the center of the ring lies a very small *m*-chromosome bivalent (*m*), which as in so many other cases is formed in the late prophases by conjugation of its two members. The thirteenth chromosome is the small unpaired univalent *s*-chromosome (*s*) which divides like all the others in the first division but passes undivided to one pole in the second division. In three of the seven males I have, this chromosome is of the same size as the *m*-chromosomes. In two individuals of the same type it is somewhat larger, though markedly smaller than the large bivalents. In the remaining two males (which constitute "Type B") the *s*-chromosome is wanting in all the cells, whether spermatogonia or spermatocytes. In these individuals the first spermatocyte division uniformly shows 12 chromosomes (Fig. 1, *c*) and the second 11, the grouping being otherwise more or less nearly similar to that in the first type.

tion probably cannot now be resolved, since the original specimens of Montgomery's material are not in existence. I think it probable that two different species have been under observation, and there is some reason to suspect that Montgomery's material may have been *Euthoctha galeator*. This case illustrates the extreme importance, in work of this kind, of preserving every individual from which cytological material is taken.

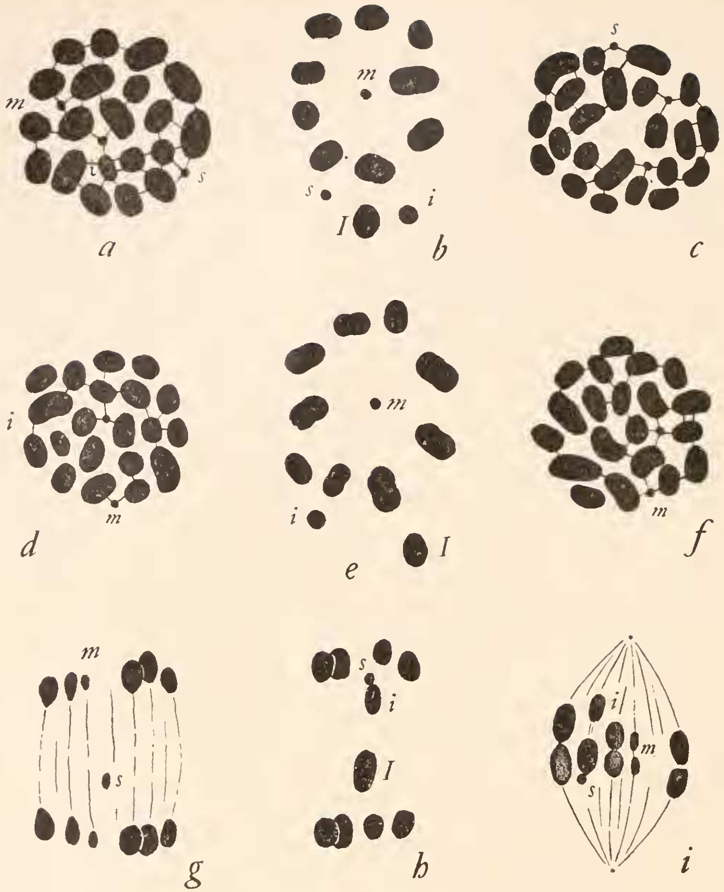

FIG. 1. *Metapodius terminalis.*[1] *a*, polar view, spermatogonial group, Type A; *b*, first maturation-division, Type A; *c*, female, Type A (probably a young follicle cell); *d*, spermatogonial group, Type B; *e*, first spermatocyte-division, Type B; *f*, female group, Type B; *g*, second division, Type A, showing *s*-chromosome free (the idiochromosomes do not appear in the plane of section); *h*, second division in side-view, *s*-chromosome coupled with small idiochromosome; *i*, second division, *s*-chromosome coupled with the large idiochromosome. (Fig. *a* is from a specimen taken at Madison, N. J.; *b*, *g*, *h*, *i*, from one individual from Charleston, S. C.; *c*, *e*, and *f* from Raleigh, N. C.; *d*, from Mansfield, Ohio. A second male of Type B was taken at Raleigh, N, C., from the same catalpa tree with an individual of Type A.)

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[1] The enlargement is 3700 diameters — somewhat less than that of the figures in my preceding papers. The figures are all from camera drawings and are not schematized, except that in Fig. 1, *g*, one pair of the chromosomes has been slightly displaced in order to show the *m*-chromosomes more clearly. No attempt is made to show details of the achromatic spindles. In all the figures *I* denotes the large idiochromosome, *i*, the small one, *m* the *m*-chromosome, and *s* the *s*-chromosome. (The latter not to be distinguished from the *m*-chromosomes in *a* and *c*.)

When the *s*-chromosome is present it sometimes (in about 20 per cent. of the cases tabulated) lies free — *i. e.*, not connected with any other in either division — and after dividing in the first division, passes undivided to one pole in the second (Fig. 1, *g*). *In most cases it is in the second division attached to one or the other of the idiochromosomes and passes with it, undivided, to one pole* (Fig. 1, *h*, 1, *i*). In either case one pole receives 11 chromosomes and one 12, as may clearly be seen in polar views of the late anaphases which show both daughter-groups of chromosomes in the same spindle. Four classes of spermatozoa are accordingly formed in this type, which correspond to those described in *Banasa calva*. Designating the ordinary chromosomes or "allosomes" as "O," the large and small idiochromosomes respectively as "I" and "i," the *m*-chromosome as "m" and the *s*-chromosome as "s" the classes are as follows:

(1) $9\,O + I + m + s = 12,$
(2) $9\,O + I + m = 11,$
(3) $9\,O + i + m + s = 12,$
(4) $9\,O + i + m = 11.$

So far this is identical with the conditions described in *Banasa calva* except that in the latter case the unpaired chromosome fails to divide in the first division but divides in the second, while the reverse condition obtains in *Metapodius*. But there is now an important difference to consider which involves the most interesting phenomenon that occurs in this form. In *Banasa calva* the four classes are equal in number. In *Metapodius*, at least in certain individuals, this is not the case; for the *s*-chromosome shows a marked tendency to couple with the small idiochromosome rather than the large, which produces an excess of spermatozoa in which these two chromosomes are associated. It is somewhat difficult to secure adequate data, since the nature of the coupling can, as a rule, only be determined with certainty in side views of the middle anaphases. Out of 34 clear cases (taken from two individuals) the *s*-chromosome is coupled with the small idiochromosome in 24 and with the large in 10 — *i. e.*, in about 70 and 30 per cent. respectively, a ratio which may very likely be somewhat altered with a larger series of data. Of the four classes, accordingly, 2 and 3 are more numerous than 1 or 4.

Turning now to the spermatogonia, we find an accurate correlation between the spermatogonial chromosome-groups and those of the maturation divisions. In all cases there are 18 equally paired larger chromosomes, an unequal pair of idiochromosomes and a very small pair of *m*-chromosomes; and these 22 alone are present in the individuals of Type B (Fig. 1, *d*). In those of Type A an *s*-chromosome is present in addition, making 23 in all (Fig. 1, *a*). In three of the five males of this type, as stated above, the *s*-chromosome is no larger than the *m*-chromosomes, and the spermatogonia correspondingly show 20 large and three very small chromosomes. In the remaining two individuals of this type the *s*-chromosome is considerably larger than the *m*-chromosomes, both in the maturation-divisions and in the spermatogonia. The spermatogonia of these individuals seem therefore, at first sight, to show 21 large chromosomes and two small. In Type B, which have but 22 chromosomes, the first maturation-division shows but 12 chromosomes, the second 11; and only two classes of spermatozoa are formed, which correspond to Classes 2 and 4 of Type A.

The foregoing data, when compared with the conditions found in the female, give a decisive result regarding the relation of these chromosomes to sex-production. If the unpaired *s*-chromosome were of the same nature as the odd or "accessory" chromosome of other coreids we should expect to find one such chromosome in the male and two in the female; and since males and females alike possess in addition two small *m*-chromosomes the males should show three small chromosomes and the females four. Such however is not the case. In both sexes there are individuals that possess three small chromosomes (Fig. 1, *a*, 1, *c*) and others that possess but two (Fig. 1, *d*, 1, *f*). Evidently therefore the *s*-chromosome is indifferent as regards the sex-characters. On the other hand, close study of the larger chromosomes shows the same relations as those observed in other forms that possess unequal idiochromosomes. In the female groups all are equally paired. In the male all are thus paired save two, one of which is evidently the small idiochromosome.[1]

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[1] This fact is not always readily made out, since the small idiochromosome is not very markedly smaller than the others; but I am sure of the observation, and the fact was determined in many spermatogonial groups long before I suspected the presence of a pair of idiochromosomes in this genus.

The usual conclusion follows that spermatozoa containing the large idiochromosome produce females and those containing the small one produce males. It is equally clear that the *s*-chromosome, though unpaired and hence a heterotropic chromosome in behavior, is not physiologically comparable to an odd or "accessory" chromosome of the usual type.

The numerical relations between Types A and B are interesting. Since in maturation the *s*-chromosome couples more frequently with the small idiochromosome (which is confined to the male) we should expect to find the *s*-chromosome in a majority of the males and in a minority of the females; and such is indeed the case in the 12 individuals that have been examined. Of the seven males, five are of Type A and two of Type B — a ratio that happens to be nearly identical with that shown in the coupling. Of the five females on the other hand, only one is of Type A (with three small chromosomes, Fig. 1, *c*),[1] while four are of Type B (Fig. 1, *f*). The number of individuals is of course too small to give an accurate result; but as far as they go the facts are in conformity with the expectation created by the mode of coupling in the spermatogenesis.[2]

BANASA.

The remarkable relations observed in *Metapodius terminalis* probably give the explanation of those I formerly described in *Banasa calva*, though I am not yet in a position to prove this positively. I have now new material of this genus from individuals ranging from New England to Arizona, and comprising both of the more frequent species, *B. calva* and *B. dimidiata*.[3] All

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[1] This individual differs from all the others in having 22 instead of 20 large chromosomes, or 25 in all. I have found a similar variation in the number of larger chromosomes in different individuals of two other species of the genus (*M. femoratus* and *M. granulosus*) as will be described hereafter. These variations appear to have no constant relation to the presence or absence of the *s*-chromosome and hence do not affect the questions here under consideration.

[2] Besides the two types of males and females described above we should expect to find a third type in each sex containing two *m*-chromosomes and two *s*-chromosomes. Such forms have not yet come under my observation, and it is possible that gametes containing both these forms of chromosomes are infertile towards each other.

[3] I am indebted to the well known hemipterist Mr. E. P. Van Duzee, of Buffalo, for the identification of these and many other species.

the new material of *calva* differs from the Long Island material that I formerly described in the *absence of the small unpaired or heterotropic chromosome*, though in every other detail they are identical. To facilitate the comparison I give three new figures from the Long Island material (which, as above stated, includes only two slides from the Paulmier collection). The first division here always shows 15 chromosomes (Fig. 2, *a*) of which two, the unpaired chromosome and the small idiochromosome, are much smaller than the others. Owing to the passage of the unpaired chromosome to one pole without division in the first maturation division the secondary spermatocytes are of two types, showing respectively 14 and 13 chromosomes (Figs. 2, *b*, 2, *c*) — a relation shown with perfect clearness in a large number of cells. In all my new material on the other hand (from New York, Ohio, Colorado and New Mexico) the chromosome groups are exactly similar to those of the Long Island form except that the small unpaired chromosome is missing. The first division accordingly always shows 14 chromosomes instead of 15, of which one (the small idiochromosome) is smaller than the others (Fig. 2, *d*). The second division always shows 13 chromosomes, (Fig. 2, *e*) of which one is a typical idiochromosome-bivalent; and in the ensuing division all the spermatids receive 13 chromosomes, half receiving the small idiochromosome and half the large. Both the spermatogonial and the ovarian groups accordingly show 26 chromosomes, the small idiochromosome being present in the male only (Fig. 2, *f*). In every respect, therefore, these individuals show the typical pentatomid relations, and agree with Type B of *Metapodius.*

Banasa dimidiata agrees essentially with this except that to my astonishment the number of chromosomes was found to be much smaller, namely, in the spermatogonia 16 (Fig. 2, *j*), in the first division nine (Fig. 2, *g*), and in the second eight (Fig. 2, *h*). It is noteworthy that these two species, which are so closely similar as sometimes to have been confused by systematists, should differ so widely in the number of chromosomes.

The difference between the material from Long Island, labeled "*Banasa calva*," and my own at first led of course to the suspicion that an erroneous identification was at fault; and this is indeed possible since the Paulmier slides were not accompanied by the original specimens. But the exact similarity of the two forms in every respect apart from the unpaired chromosome, and my failure to find any other similar form in an examination of nearly all the species of Pentatomidæ that might be confused with this species, leads me to believe that the case of *Banasa* *calva* is probably similar to that of *Metapodius terminalis*, the Long Island form being of one type (corresponding to Type A of *Metapodius*, with an unpaired *s*-chromosome) and the others of the other type (corresponding to Type B of *Metapodius*). In *Metapodius* the two types occur side by side in the same locality ;

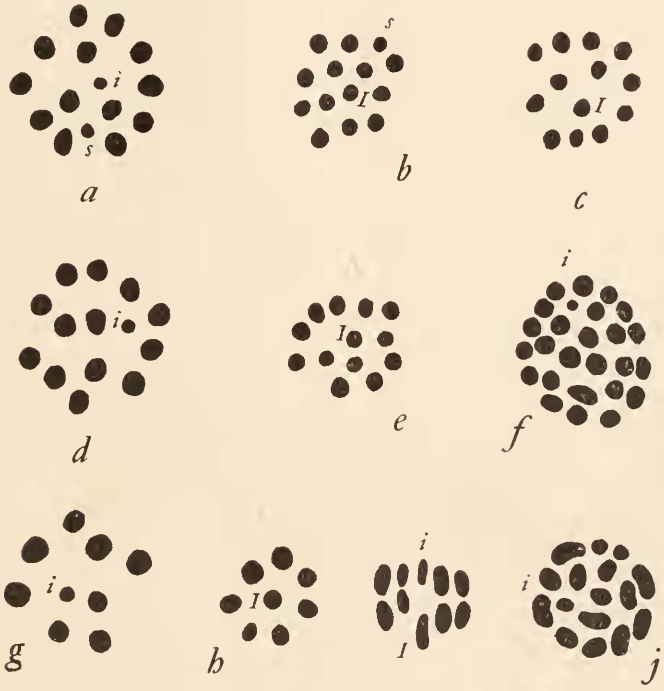

FIG. 2. *Banasa*. (*a–f*, *B. calva*, *g–j*, *B. dimidiata*.) *a*, Long Island form (Paulmier), first spermatocyte-division ; *b*, 14-chromosome type of second division ; *c*, 13-chromosome type, from the same cyst ; *d*, western form (Colorado), first spermatocyte-division ; *e*, second division of same ; *f*, spermatogonial division ; *g*, *B. dimidiata*, first division ; *h*, second division, polar view ; *i*, side view of same ; *j*, spermatogonial group. (In *a* it is impossible to distinguish between the *s*-chromosome and the small idiochromosome.)

and the same should be true of the Long Island *Banasa calva* if the two cases are really alike. But this question can only be settled with additional material. All the individuals from western New York (Buffalo) and further west are of Type B. It therefore seems not improbable that one of the types has been lost in the western forms, or conversely, that Type A has been added to B in Long Island, perhaps in a local colony or variety.

Comment.

The strong support lent by the foregoing facts to the general theory of the individuality — or, as I should prefer to say, the genetic identity — of the chromosomes is so obvious as hardly to require comment. I will here only call attention to the interest of the coupling of the *s*-chromosome with one of the idiochromosomes in *Metapodius*. This phenomenon is doubtless comparable in a general way to the coupling of the true odd or "accessory" chromosome with one of the ordinary bivalents first briefly recorded by Sinéty[1] in the Phasmidæ, and carefully studied in several of the grasshoppers (in some of which the facts are more complicated) by McClung[2] who has given an interesting discussion of the subject. I have observed chromosome-couplings in four families of the Hemiptera heteroptera and believe the phenomenon will be found to be of wide occurrence in the insects, and perhaps in other animals. It seems well within the bounds of possibility that such chromosome-couplings may give the physical basis of certain forms of correlation in heredity. If the chromosomes embody the primary factors of heredity (the working hypothesis upon which I am proceeding in these studies), it must no doubt be assumed that each chromosome contains the determinants of many characters; and the association of such determinants in the same chromosome may imply the constant correlation of the corresponding characters in heredity. But in addition to this, certain correlations, such as are observed in some forms of hybrids, might also be a result of a more or less pronounced tendency of certain chromosomes to cohere in a definite way, so as to be more frequently or even invariably

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[1] *La Cellule*, XIX., 1901–1902.

[2] Biol. Bull., IX., 5, October, 1905.

associated in the germ-cells. In the case of *Metapodius* such a tendency is shown in the more frequent coupling of the *s*-chromosome with the small idiochromosome, which leads to its more frequent passage to the male-producing pole, and hence to its more frequent appearance in the male. This reminds us of certain crosses of Lepidoptera observed by Standfuss,[1] and more recently by Doncaster and Raynor,[1] in which there is a tendency for a particular set of specific or varietal characters to appear more frequently in one sex than the other. Thus, in *Abraxas*, as reported by the last named observers, after crossing the original form (*A. grossulariata*) with an albinistic variety (*lacticolor*) to which it is dominant, the cross ♂ DR × ♀ RR gives both sexes of both forms, but the reverse cross ♀ DR × ♂ RR results in a sharp separation of the sexes of the two forms, all the resulting males being DR and the females RR. This, as the authors show, may be explained by the assumptions, first, that the sex borne by the egg is uniformly dominant (as appears to be the case in the Hemiptera) and second that the dominant somatic character (*i. e.*, the *grossulariata* pigmentation) uniformly couples with the male character in the egg while in the spermatozoon no coupling occurs. Such a chromosome-coupling as that observed in *Metapodius terminalis* gives a very definite basis for the possible explanation of couplings of the sexual characters with specific or varietal ones; and it seems possible that we may in this direction find a means of testing decisively the whole chromosome-theory of heredity. In the case of *Metapodius* I have not thus far been able to find any constant differences between individuals of Types A and B; but only the external characters are available for examination. I hope hereafter to examine this question more thoroughly, both in *Metapodius* and in *Banasa*.

Zoölogical Laboratory, Columbia University,
February 4, 1907.

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[1] See Castle, *Bull. Mus. Comp. Zool.*, XL., 4, 1903.

[2] *Proc. Zool. Soc. London*, June 7, 1906.

SPAWNING BEHAVIOR AND SEXUAL DIMORPHISM IN FUNDULUS HETEROCLITUS AND ALLIED FISH.

(Contributions from the Zoölogical Laboratories of the University of Michigan. No. 108.)

H. H. NEWMAN.

Introduction.

Of recent years much stress has been laid upon the structural basis of behavior, especially among the lower organisms. Among the higher animals, on the other hand it has long been understood that function and structure are simply dynamic and static phases of the same thing. There would be little excuse for the present paper, then, unless it should serve to show that these ideas of structure and function — sexual dimorphism and spawning behavior — have a far wider application than has commonly been supposed. It will be shown that even minute, temporary structures, that have previously escaped the eye of the investigator, are as truly adaptations for spawning as are the more obvious secondary sexual characters, such as differences in the sizes of fins, in color pattern, in body form, etc.

My attention was first called to this subject by chance. One day early in the summer of 1906, while engaged in cross-breeding species of *Fundulus* at the Marine Biological Laboratory of Woods Hole, I was fortunate enough to observe the spawning act in the species *Fundulus heteroclitus*. These fish were spawning in a small aquarium and in a good light so that the entire process could be observed in minute detail without difficulty. Afterwards I was fortunate in being able to observe the spawning of *Cyprinodon* under equally favorable conditions. These observations led to a closer study of the behavior of these species and to a consideration of their sexual dimorphism as the structural basis of this behavior. The other two available species of *Fundulus*, *F. majalis* and *F. diaphanus* were then brought in for purposes of comparison.

The wash drawings reproduced in Plates XXVII. and XXVIII. were made under my direction by Miss Ella Weeks of the State Agricultural College of Kansas. I take this opportunity of expressing my appreciation of the quality of her work and my indebtedness to her for her service. I also wish to express my thanks to Professor Jacob Reighard for his helpful suggestions and criticism.

The plan of the paper is to treat each species separately, to give a summary of the main points, and to conclude with a general discussion of the origin and significance of the structure and behavior described.

The four species dealt with all belong to the family Pœciliidæ (the killifishes). The following statements referring to sexual dimorphism in this family are quoted:

> "Sexes usually unlike, the fins being largest in the males, but in some species the females are much larger in size. Many of the species are ovoviviparous, the young well developed at time of birth. In these species the sexes are very unlike, the anal fin of the male being developed into an intromittent organ."—Jordan and Evermann, "Fishes of North America," p. 631.
>
> "In many species the sexes are dissimilar, the female being larger and less brilliantly colored, with smaller fins."—"Cambridge Natural History."

I have been unable to find anything in the literature concerning the spawning behavior of these species.

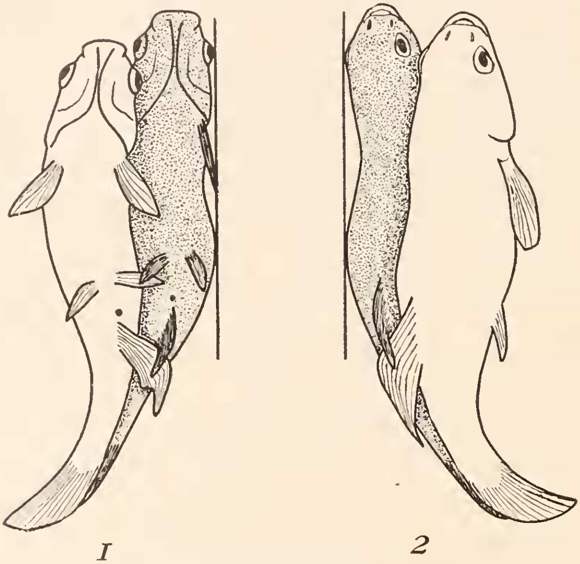
For purposes of clearness it seems best to present the facts on spawning behavior before those on sexual dimorphism, since behavior throws so much light on the significance of structures.

FUNDULUS HETEROCLITUS (Linn.).

(Common Killifish; Mudfish; Cobbler; Mud Dabbler; Mummichog.)

Spawning Behavior.

The spawning act proper was the first to gain my attention and it is hardly likely that I should have noticed it had I not been slightly familiar with Professor Jacob Reighard's unpublished observations on the spawning habits of certain inland species. In spawning the male clasps the female firmly around the slender portion of the body just back of the dorsal and anal fins, using as claspers his large, strong anal and dorsal fins. The two fins of the male slip in under the homologous fins of the female, which are raised up to admit them, and nearly surround the body of his mate. The ventral fins of adjacent sides are also locked in similar fashion. The female is usually forced against some perpendicular solid object such as a stone, a mass of seaweed or the glass sides of the aquarium — most frequently the latter when in captivity. When the female is thus seized by a male she suddenly assumes a characteristic attitude, the whole body, when observed from above having the conformation of a somewhat flattened S, the head being pressed against the solid, that part of the body just back of the head being bowed outwards and not touching the solid surface, the region of the abdomen being again against the solid, and finally the whole tail region being free from the solid and bent sharply away from it and slightly upwards (see Text Plate I.). In this rigid position she is supported from the bottom by means of her anal fin, which



Text Plate I. Showing the spawning attitude of male and female Fundulus heteroclitus, the female darkened.

Fig. 1. Ventral view, showing the position of anal and ventral fins.

Fig. 2. Dorsal view, showing the position of dorsal fins. This view is a half side view. The straight line indicates the points of contact with a solid substance such as the glass sides of the aquarium. ½ natural size.

extends nearly at right angles from the belly and is thick and stiff and well adapted for supporting the weight of the two bodies and often becomes much inflamed by frequent contact with the bottom. The male, clasping the female firmly as he does, holds his entire body against hers for from one to two seconds. While in this position a quivering vibration of the posterior half of the bodies of both fish occurs, during which eggs and sperm are extruded in very intimate contact with each other. Whether this vibratory movement is initiated by one or both sexes I cannot say for certain, but I am inclined to believe that the female only is responsible, the male simply remaining passive and taking up the vibration from the female. My reasons for believing that such is the case are: first, the vibratory rhythm of the two fish spawning together is always in perfect unison, which would hardly be the case when two fish of very different sizes clasp; second, I have often seen females assuming the spawning attitude and going through the vibratory movement when there were no males in the vicinity.

This spawning act is highly adaptive in several respects:

1. The vibratory movement serves to extrude ripe eggs and ripe sperm at the same instant. It is only in this way that the sexual products can be extruded as is shown by the fact that the females are forced to adopt this method of relieving themselves when there are no males present.

2. The position assumed by the two sexes is such that eggs and sperm are extruded in very close proximity and could scarcely fail to come in contact. Chances of failure are minimized by the fact that on the one hand the milt is shot out with considerable force directly at the eggs as they are extruded, and on the other the eggs exert a strong chemotactic influence upon the sperm, which I demonstrated by the following experiment. A freshly stripped egg was put on a glass slide with a few drops of water and then a drop of sperm was put in the water at a distance of half an inch. In a very short time the egg was surrounded by a dense cloud of sperm and inside of two minutes all of the sperm was seen to be gathered about the egg. In addition to this the surface of the egg is very glutinous and probably holds all sperm that comes in contact with it.

3. The position of the anal fin of the female is such that it supports the weight of both fish, the distal end of the fin being in contact with the bottom which under natural conditions is usually composed of soft mud. Since this fin receives the vibratory movement of the body it necessarily stirs up a considerable amount of mud and also makes a shallow depression. The eggs, as soon as they are extruded, fall to the bottom and either settle in the small hole or are at least partially concealed by the settling mud. That some such protection is necessary is shown by the fact that the fish themselves are very fond of their own eggs, devouring them eagerly when they can be seen. This curious type of cannibalism was observed repeatedly in aquaria where the bottom was free from mud. The females were the chief offenders in this respect although young males occasionally devoured the freshly extruded eggs. It was a very common sight to see other females rush up and seize the eggs as soon as they were extruded. Frequently, however, females were observed to turn and devour their own eggs. This destruction of eggs probably occurs only to a very limited extent under normal conditions for the reasons cited above.

Courtship, Rivalry and Display.

As might be expected, the male takes the more active and aggressive part in courtship but the female frequently displays herself in such a way and assumes such postures as are calculated to attract the male. Females laden with very ripe eggs frequently display themselves by turning on their sides near the bottom, and spurning the latter with their tails, thus causing their silvery white bellies to flash in the light. This, I noticed, seldom failed to attract the males if there were any of the latter about. This curious flashing movement is by no means restricted to the females of this species, but was observed occasionally in the males of the same species, in both sexes of *Fundulus majalis*, and in several other marine species. It was, however, especially noticeable in actively spawning females and seems to be of undoubted service in attracting males.

When both sexes are at the height of their sexual activity there is little that could be termed courtship. The females are burdened with their great masses of eggs, that must distend their adbomens uncomfortably, and are eager to get rid of their burdens. This can most easily be accomplished by contact with the males, but may be and frequently is accomplished without the latter. The females usually, however, retire to the bottom and place themselves in contact with some solid object, sometimes only the snout being actually in contact, thus assuming a position in which they may be most readily clasped by the male. Whenever a male sees a female in this position he loses no time in spawning with her. If a female after taking up the position just described is not joined by a male she is very apt to relieve herself by assuming the S-shaped posture characteristic of the spawning act and by vibrating her body just as she would if clasped by the male. The eggs are thus plentifully extruded, but if no males are present, they are not and probably never can be fertilized. If, however, any males are in the vicinity they are always attracted by the vibratory movement and dart toward the source of vibration.

Courtship, if such by courtesy it may be called, occurs shortly before the sexual climax. Fish in this condition swim about comparatively quietly in pairs, the female above and the male just below and slightly back of her. This position enables him to see her more readily and at the same time to guide her about by gently butting her on one side or the other with the top or sides of his head. I have observed very many pairs swimming about in this way for considerable periods of time. Gradually the male becomes more excited in his movements and the preliminary courtship merges into spawning behavior proper. At first the attempts at spawning on the part of the male are apt to consist merely of efforts to corner the female and to induce her to seek some retired spot at or near the bottom. To accomplish this he rises from beneath her and butts her downwards with the sides of his head. If he succeeds in driving her into a suitable place he attempts to spawn with her, the first few trials lacking the vigor characteristic of ruling males. Sexual excitement increases rapidly so that before many minutes have passed the male is apt to be seen spawning promiscuously with any female that he encounters. This transition from courtship to spawning was observed both in aquaria and under natural conditions. In the open, excited males were often seen to be in rapid pursuit of the females, succeeding occasionally in cornering and clasping them. The pursuit of the males was often so impetuous that the females were entirely frightened away. The coyness on the part of the females acts as an excitant on the males.

Rivalry among the males is very keen and as a rule those whose "spawning plumage" is most brilliant succeed in driving away all competitors. I have observed under normal conditions that a certain male, always the most brilliantly colored one in the neighborhood, seems to control the situation, driving away all males that attempt to encroach upon his territory. The size of the male seems to be a much less important factor in determining his success than is the degree of sexual maturity as indicated by the brilliance of his coloration, for I have often seen a male of comparatively small size put to rout several others of twice or three times his size, such is the impetuosity of his attack. I was able to observe this rivalry to better advantage in aquaria where it was possible to identify the various individuals and thus to keep an accurate record of the success or failure of each male. The following record was made on June 20, 1906.

A fresh lot of *F. heteroclitus* was placed in the aquarium early in the morning. At about ten o'clock in the forenoon they were observed to be actively spawning. About twenty females were spawning on the bottom of the aquarium on the side away from the light. Seven males seemed ready to take part in the process, but one very large male, more brilliantly colored than the rest, continually drove away all other males that attempted to spawn. So solicitous was he about driving off his competitors that he could scarcely attend to all of the females that were ready to spawn. While the ruling male was engaged in clasping one female another male in a remote portion of the aquarium would occasionally succeed in spawning with another female. Whenever such an occurrence was observed by the ruling male, he always gave chase and invariably routed the intruder. This male was so much larger and more vigorous than any of the others that none dared to dispute his authority to the extent of offering battle. For purposes of experiment I removed this male from the aquarium and put him in a smaller vessel by himself, being careful to keep him out of the strong light. When thus removed from the sexual environment, he almost immediately underwent a very marked change in appearance, becoming decidedly lighter in color, and inside of ten minutes losing all of the steely blue glint that is so characteristic of sexual excitement. Putting a dark colored fish in a strong light causes a similar lightening of color, but that the change in question was not due to light seems certain for all strong light was excluded. As soon as this ruling male had been removed the other males usurped his prerogative and a struggle for supremacy immediately ensued. The combat between the six remaining active males was for a time very evenly waged, since there was no great disparity in size, but after about ten minutes a single male, and he not the largest, had gained supremacy and had succeeded in driving away his rivals whenever they approached. Occasionally one of the outsiders plucked up sufficient courage to challenge the ruling male and a combat ensued. The males fight with their heads and mouths, butting one another fiercely and accasionally locking jaws and struggling like dogs. When a male wishes to challenge he approaches rather cautiously, body trembling with excitement and all fins extended to the utmost, presenting as formidable an aspect as possible. The male thus challenged adopts a similar attitude and rushes at his foe with alacrity. Curiously enough the male that has once gained supremacy always emerges victorious from these contests, and the defeated male retires into hiding until he has regained sufficient courage to challenge again. After about half an hour the large male that had been removed to another vessel was returned to the aquarium. At first he seemed to take no interest in the spawning activity going on about him, but gradually he aroused himself and made an occasional half-hearted attempt to clasp a female that came near him and advertized by her attitude her desire to spawn, but he was always rudely interrupted and put to rout by the new ruling male, which although of very much smaller size, attacked with such vigor that his much bulkier opponent was forced to retire. By degrees, however, the large male increased in vigor, at the same time growing darker and reassuming the blue glint that he had so quickly lost. In about twenty minutes he was as dark and as brilliant as before and had succeeded in ousting the usurper from his domain, although not without repeated struggles in which his victory became more decisive each time. The next day the same male was in control, but on the following day another male of medium size had acquired supremacy. It is probable that the period of sexual climax is of short duration, not exceeding three or four days. A male at the very height of his sexual activity is afraid of nothing and is practically invincible.

This account will serve as a sample of the scenes observed repeatedly in the aquaria and in natural conditions during the months of June and July. The last recorded observation of spawning in this species was taken on July 7, although ripe males and females were found for at least two weeks later.

This account of the spawning behavior of *Fundulus heteroclitus* may well be concluded with an account of a few more experiments and some additional isolated observations.

Experiment 1. — A considerable number of actively spawning males and females were separated into two aquaria, the males in one and the females in another. Inside of about fifteen minutes the males had all become nearly as pale as the females and spent their time in wandering about uneasily as though seeking for a place of escape. The females, on the other hand, seemed to be very little affected by the absence of the males but went on extruding eggs as freely as if the males had been present. It is probable that the initial stimulus to egg extrusion given by the males lasted some time after the removal of the latter. The eggs were always eagerly devoured either by the female that laid them or by another that rushed up and siezed them before she could turn around. It is hardly probable that eggs are so eagerly devoured in the open, as the food supply is not restricted as in an aquarium.

Experiment 2. — An aquarium was prepared with mud and stones on the bottom to approximate natural conditions, and in it were placed five spawning fish of each sex. These fish, instead of appearing to enjoy their new surroundings, lost all interest in spawning and spent all of their time in exploring their environment. In the meantime the males lost all of their "spawning plumage" and became decidedly pale. The females too seemed to have forgotten about spawning in their anxiety to become familiar with the new neighborhood. On the following day the fish were still uninterested in spawning and I concluded that they had passed the sexual climax while they had been busy with their explorations.

Experiment 3.—I put several males that had been isolated for several days into an aquarium containing only females. These had been extruding eggs at intervals, but as soon as the males appeared, they seemed to become excited and immediately began to take up spawning attitudes and to extrude eggs in much increased amounts. It seems certain that the presence of the male, even when the latter refrains, as in this case, from any participation in the spawning act, exercises an exciting influence upon the female. The stimulus is probably a visual one, for the appearance of the male is very characteristic.

Additional observations:

1. Occasionally pairs were observed to come together and spawn in open water without being in contact with any solid. It was also not unusual to see them spawning against the bottom instead of against some more or less perpendicular object.

2. On three occasions I observed a female following a male around and apparently endeavoring to incite him to spawn with her by bumping him and placing her body in contact with his. On one occasion she succeeded in inciting him to clasp her for an instant. This assumption of the initiative on the part of the female struck me as being decidedly abnormal and may have been a perversion of instinct, due to confinement.

3. I observed that females that were being guided about by the males occasionally seemed to resent this infringement upon their liberty and engaged in a somewhat mild form of contest with the males, returning their butting in kind. The male, however, invariably seemed to have his way in the end.

4. On June 27, I observed *F. heteroclitus* spawning in the Eel Pond in the shadow of a boat and in about eighteen inches of water. The males would chase females out beyond the shadow but usually returned quickly to the shade. I have noticed repeatedly that the fish prefer the darker places for spawning.

5. After watching the spawning of this species in the open, I believe that large males, when at the height of their sexual period, control considerable areas in the Eel Pond and elsewhere. Although an active male may pursue a female or another male for considerable distances he soon returns to the neighborhood over which he seems to exercise authority. This phenomenon is by no means unusual in fish.

Sexual Dimorphism.

The following passages, refering to sexual dimorphism, are selected from Jordan and Evermann's systematic account:

> ". . . fins moderate, the dorsal inserted in males midway between snout and tip of caudal; in females farther back; oviduct attached to anterior ray of anal fin for one-half to two-thirds its length; . . . Coloration in males dark dull green, the belly more or less orange yellow; sides with numerous quite narrow, ill-defined silvery bars made up of silvery spots, most distinct posteriorly; besides these are numerous conspicuous white or yellow spots, irregularly scattered; vertical fins dark with numerous small round pale spots; dorsal often with a blackish spot on its last ray; anal and ventrals yellow antertiorly; under side of head yellow; young males with alternate bars of dark and silvery, the former becoming in time the ground color, the dorsal ocellus more distinct. Females nearly plain olivaceous, lighter below, without spots or bars, the scales finely punctate; sides often with about fifteen dark crossbars or shades. Young, especially young females, with more or less distinct dark cross bands; these always present in the very young, in females narrower than the interspaces, in males much broader and less numerous."

This description, while accurate enough so far as it goes, needs to be supplemented with regard to certain details. It also fails to take into consideration the fact that there are marked seasonal changes not only in color but in the actual size of certain parts such as belly and fins, and in the production of certain temporary organs in the male.

First of all I would like to supplement Jordan and Evermann's account and to call attention to certain points. Then I shall be in a position to discuss the seasonal changes.

The description of the male is fairly accurate for one out of the breeding season. I wish, however, to call attention to the relatively large size of the dorsal and anal fins of the male as compared with those of the female (see Plate XXVII., Figs. 1 and 2).

The enormous difference in the fin coloration of the two sexes should also be emphasized, those of the female being almost devoid of pigment while those of the male, although more deeply colored in the spawning season, are always markedly pigmented, the pigment being laid down is such a way as to produce a mottled pattern. The posterior and proximal half of the dorsal is, however, always much darker than any other area on the fins and is the equivalent of the much more distinct spot seen on the dorsal of male *Fundulus majalis*. There is also a marked difference in the shape and in the strength or stiffness of these fins in the two sexes. In the male there is a pronounced posterior prolongation of both fins, especially the anal. These fins are stronger and better provided with muscle in the male than in the female and hence are better fitted for clasping organs.

Discussion of the Seasonal Changes.

The seasonal changes may be classified as follows:

In the female:

1. Paling of the general body coloration.

2. Distension of the abdomen with eggs and consequent lessened activity.

3. Thickening and inflammation of the anal fin.

In the male:

1. Intensification of pigmentation in definite regions.

2. Acquisition of a steely blue gleam in the scales of certain regions. (1 and 2 are spoken of collectively as "spawning plumage.")

3. Development of certain temporary organs on the scales that I have chosen to call "contact organs."

1. The paling of the general body coloration in the female and the intensification of pigmentation in the male might be attributed to the opposite metabolic conditions prevailing in the two sexes at this period. The female, having to sacrifice so much of her vitality for egg production, must have a lowered somatic metabolism, the index of which is the diminished production of pigment. The male, on the other hand, seems to have much excess vitality, since the production of sperm is far less taxing on somatic vitality than is the production of eggs. The deposition of pigment here

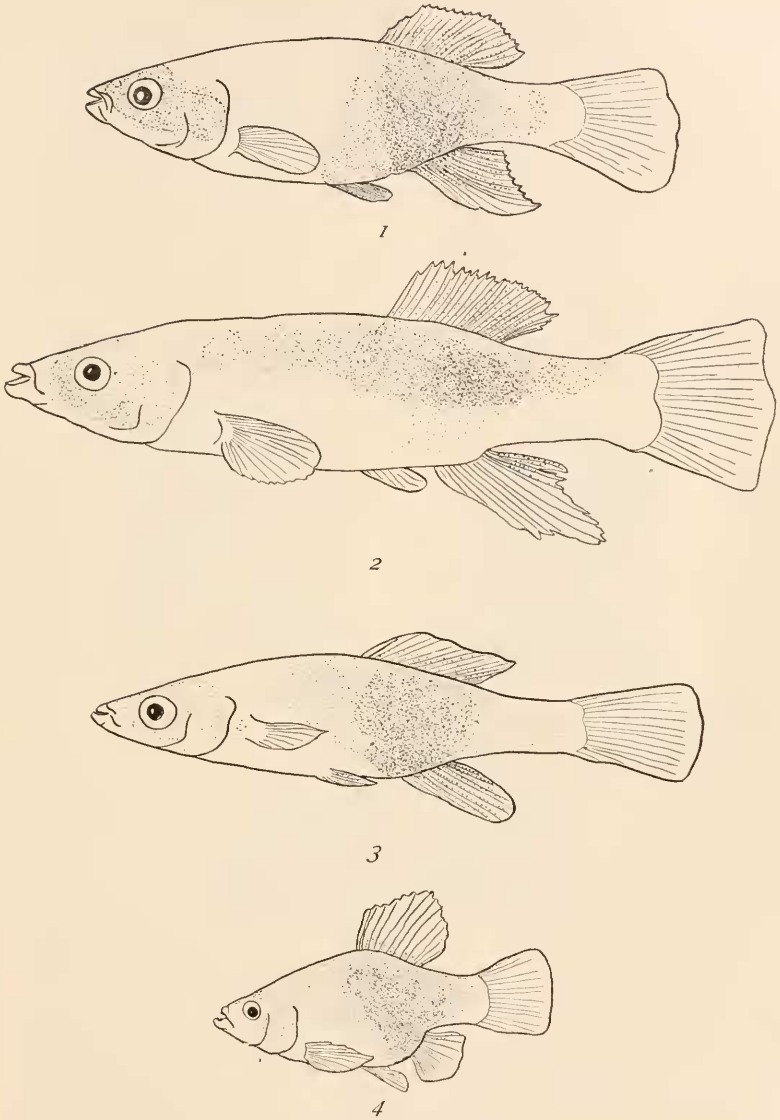
is probably an index of a very rapid metabolism that expresses itself in other ways such as greater activity, greater courage, and in the production of excrescences, etc.

2. The thickening of the anal fin in the female may be partially a phenomenon of inflammation produced by irritation. The sources of irritation are twofold. In the first place the tip of the fin is rubbed violently against the bottom during the spawning act. In the second place the frequent expulsion of eggs through the tubular extension of the oviduct that runs down the posterior ray of the fin, probably causes inflammation in this and adjacent parts. The thickening of the fin gives a firmer support for the spawning pair than would the fin in its usual condition, and in addition stirs up the mud more effectively as has been shown.

3. The steely blue gleam so characteristic of spawning males, reminds one of iridescence but is not of the same character. The blue color is probably due to a combination of chromatophores and iridicytes. The former are extremely extensible in that the pigment is capable of flowing out over a wide area through slender, branching canals. The latter are extremely minute prismatic crystaline bodies that serve the purpose of refracting the light. They evidently lie above the chromatophores which furnish the absorbing background. In some way the colors at the red end of the spectrum are absorbed by the melanin and the combined colors of the violet end of the spectrum are reflected as the steely blue gleam. The extension and contraction of the melanophores seems to be a reflex closely associated with sexual excitement, and may be considered as a sort of involuntary flush.

4. The structures that I have chosen to designate as contact organs, occur as finger-like processes on the margins of all the scales in certain regions, and upon the fins that are used in clasping. The appearance of these processes is well shown in the photographs reproduced in Text Plate III.

It is of interest to note that contact organs occur only on actively spawning males and only upon those parts that are in contact with the female during the spawning act and upon the top and sides of the head, parts that are used for butting the female in courtship and one another when fighting. The distribution of the contact organs in the four species studied is represented in

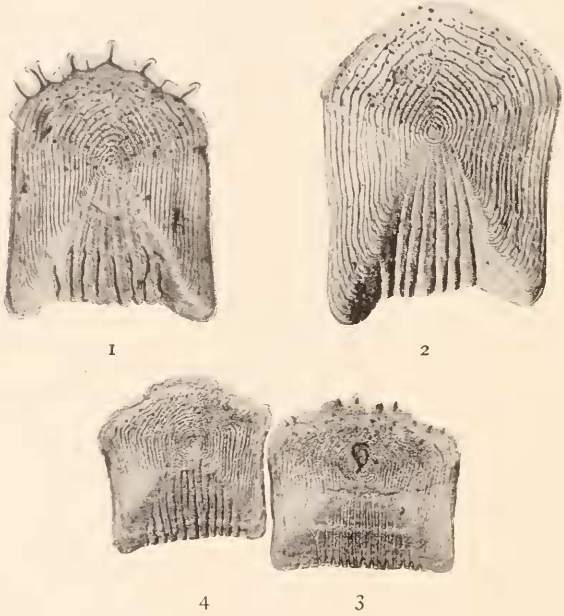


Text Plate II.

Showing the distribution of contact organs in males of the four species. The stippled areas represent regions supplied with contact organs. Where the stippling is heaviest the contact organs are most numerous and best developed. The dots on the fins represent contact organs.

1. *Fundulus heteroclitus.*
2. *F. majalis.*
3. *F. diaphanus.*
4. *Cyprinodon variegatus.*

Text Plate II. It is also interesting to note that the best developed and most frequent contact organs occur where the pressure in spawning must be the greatest, namely between the dorsal and anal fins and on these fins at or near their bases. In each case the maximum distribution seen in the males collected is shown in the figure. The stippling is closer in regions where the contact organs are thickest and best developed, and more open where the latter are more scattering and less well developed. No attempt has been made in these diagrams to represent actual numbers or sizes.



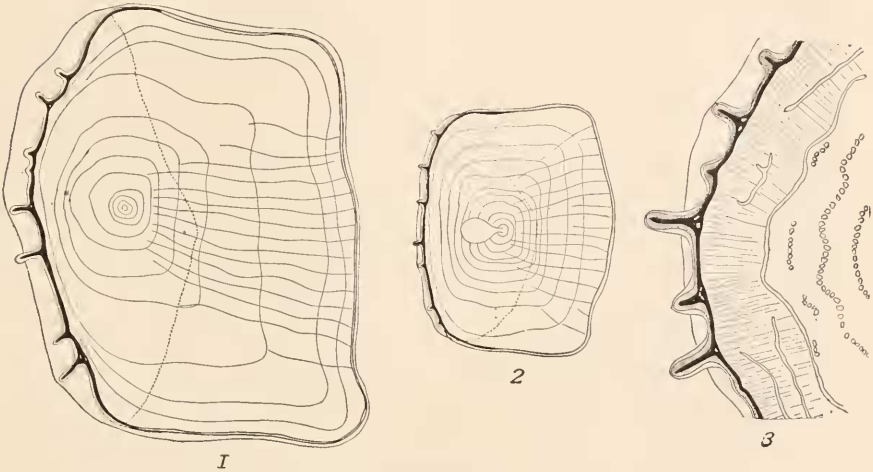
Text Plate III.

Showing scales with contact organs, × 12.

1. Scale from the side of a large male *F. majalis*.
2. Scale from a corresponding part of the body of a large female *F. majalis*.
3. Scale from the lateral line region between the dorsal and anal fins of a spawning male *Cyprinodon variegatus*.
4. Scale from a similar location of a spawning female *Cyprinodon variegatus*.

An examination of a considerable number of males reveals the fact that only those in spawning plumage possess contact organs, while some of these possess them only on the top or sides of the head. It is my belief, although I am not at present in possession of any direct evidence on the subject, that the organs are developed antero-posteriorly, either in response to a fixed law of development or because there is an earlier need for these structures on the cheeks and on the top of the head, since these parts are used in the preliminary courtship, coming into frequent contact as they do with the body of the female.

The contact organs are practically alike in all of the species studied, although they vary slightly in form and in size relative to that of the scale on which they occur. They are best developed in *Fundulus majalis*, but are simply larger here than in the species under consideration. Photographs and camera drawings (see Text Plates III. and IV.) show clearly the appearance of the contact organs in typical scales taken from males of the four species.



TEXT PLATE IV.

Camera drawings of typical scales from the region between the dorsal and anal fins of spawning males.

1. *Fundulus heteroclitus.*

2. *F. diaphanus.*

3. Enlarged detail drawing of a portion of the exposed margin of a scale taken from the side of a male *F. majalis.* The black region represents the horny margin of the growing region of the scale that is prolonged into spikes that support the contact organs. The stippled area represents the dermis. The clear outside area represents the epidermis. The striated portion is the non-calcified portion of the scale.

They consist first of a core of horny material or sclerified connective tissue (see Text Plate IV., Fig. 3), that arises like a sharp spike from the free growing edge of the scale. This skeletal support is represented in black in the drawing although in life it is nearly transparent. Various stages in the development of the spikes may be seen both in the detail and in the general drawings. They first appear as slight outwardly directed folds of the edge of the growing region of the scale, and gradually assume the spike-like form. Outside of this horny support there is a fairly thick layer of dermis, represented in the detail drawing in stippling. The histological characters of this layer I have been unable to make out in the formalin preserved specimens that have been my only resource in the present paper. Outside of the dermis there is a thin layer of epidermis that is often found worn off at the tips of the papillæ, allowing the horny spike to protrude.

The contact organs do not lie flat against the body of the fish, but stand out at an angle of about thirty degrees, so that they can readily be seen in profile with the naked eye. This attitude is decidedly advantageous for giving a rough surface or for a sensory function. Probably the former function is the principal one, although I am not sure that the latter function is not subserved. If the contact organs should prove to be sensory we can understand how their stimulation by the vibration of the female during the spawning act might account for the extrusion of sperm on the part of the male. These points have not been made out on account of the lack of histologically fixed material, but a study of the histology and function of these organs will furnish material for a more special paper.

It should be stated that the contact organs are possibly related to the so-called "pearl organs" found in other species of fish. Their structure, however, is entirely different in that they are chiefly dermal in origin and possess the horny spike-like support, while pearl organs are little more than epidermal callouses.

The resemblance of the contact organs to the teeth on the margin of ctenoid scales will probably strike the reader. It has occurred to me that here we have the origin of the ctenoid type of scale. Ctenoid scales are found on the most highly specialized of our Teleosts but are described as being absent in more primitive families such as the one with which we are dealing. Is it not possible that we have in this family ctenoid scales developed as mere temporary structures, used only by the males during the spawning season? If we admit the possibility of this condition we can see how such structures might become permanent, be produced in both sexes and subserve another function.

Fundulus Majalis.

(Killifish; Mayfish; Rockfish.)

Sexual Dimorphism.

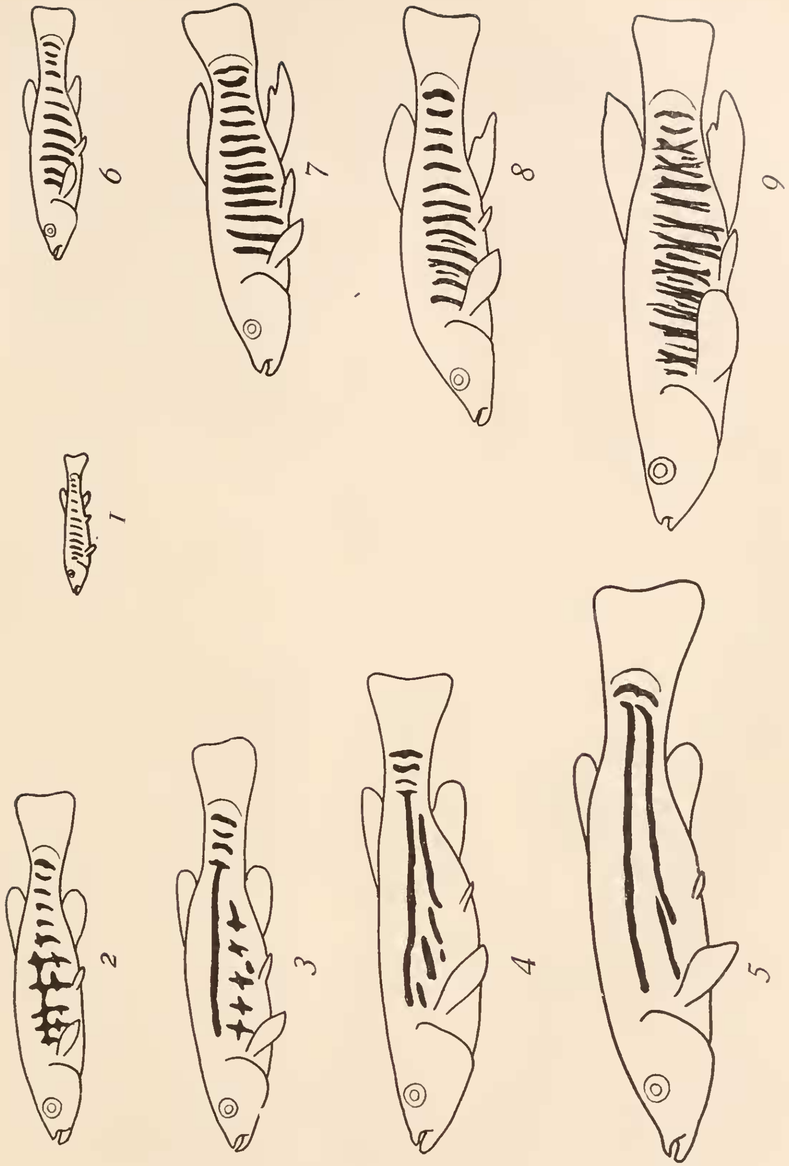
The following passages, referring to sexual dimorphism, are selected from Jordan and Evermann's account:

"... anal fin very high in males, moderate in females; ventrals long in the males, reaching past front of anal; ... Males dark olivaceous above; sides silvery or somewhat golden, with about a dozen broad transverse bars of the color of the back; posterior part of the dorsal fin with a black patch; fins yellowish or pale. Females olivaceous above, white below, a narrow black longitudinal stripe along sides about on the level of the eye and as wide as the pupil; below this two similar black stripes anteriorly and one posteriorly, the upper one being interrupted; one or two black bars at the base of caudal. Females usually larger than the males. A large male of this species, in high coloration, taken at Beaufort, N. C., showed the following colors in life: Back olive, sides and belly bright salmon yellow; lower fins clear yellow; pectorals and anals with some dusky; posterior edge of caudal dark; dorsal nearly all black, a large black ocellated spot on the last rays; opercles and under parts of head with an inky suffusion; cheeks, top of head, and mouth bronze yellow; sides with about eighteen narrow, dusky vertical bars."

The "high coloration" referred to in the Beaufort male is undoubtedly the "spawning plumage" and, although a somewhat more highly colored condition is described than I have observed in any male at Woods Hole, the account is probably entirely accurate for the species in more southern waters. The males that I have observed (see Plate XXVII., 4) have the back and the upper part of the sides almost black instead of olive as described; lower part of the sides and belly orange or golden yellow instead of salmon yellow; dorsal, anal and caudal fins golden with a tendency toward dusky, the dorsal being only a trifle darker than the other fins instead of being black as described above;

cheeks top of head, etc., heavily shaded with a bluish-black coloration, reminding one of the blue glint of the male *F. heteroclitus* instead of bronze yellow as described. The black spot on the posterior rays of the dorsal may, as in the illustration (Plate XXVII., 4), be composed of a series of spots arranged in a sort of circle. This is more apt to be the case in very large specimens.

The color pattern of both sexes is gradually modified during the lifetime of the individual and all stages in the production of the complete adult pattern are readily found. The young of both sexes are always cross barred somewhat like the adult male, but the bars are less numerous, numbering seven to ten as compared with from fourteen to twenty in adults. The increase in number of bars takes place by means of a longitudinal splitting of individual bars and by the appearance of small new bars between the old ones (see Text Plate V., Figs. 8 and 9). In the former case the two bars produced by the division of one simply spread apart and at the same time broaden out; in the latter case the small alternate new bars merely increase in size until they become nearly as large as the original bars, although it is usually easy to distinguish the latter by their greater length. In one unusually large male I observed fourteen well developed bars nearly all of which had begun to split at the ends as though preparing to double the number of bars once more. As a rule the most anterior bars are the first to show signs of splitting, the tendency proceeding antero-posteriorly. It seems to be a very general rule that meristic changes of this sort proceed in this direction. The color pattern of the females is at first similar to that of the young male, but during the second season, probably, a marked change begins to take place. The eight or ten bars that exist at that time show decided irregularities in outline, each bar, beginning with the most anterior, sending out at two places forward and backward processes, which, on examination, prove to be arranged in two longitudinal lines, the upper one on a line with the eye and the lower one on a line with the angle of the operculum (Text Plate V., Fig. 2). The processes especially those of the upper bar, continue to elongate anteriorly and posteriorly until those of adjacent bars fuse together into a continuous longitudinal stripe, the remaining portion of the bars becoming attenuated and



TEXT PLATE V.

Showing stages in the development of the adult male and female color pattern in *Fundulus majalis*. These are camera drawings made from formalin preserved specimens except in two cases 1 and 9 which are taken from free-hand drawings of living fish. 1½ natural size.

1. A young fish of undetermined sex, showing the type of pattern seen in all young fish.

2 to 5. Stages in the development of the female color pattern. The gradual change from cross bars to stripes is clearly brought out.

6 to 9. Stages in the development of the most specialized male color pattern seen in 9. Not many males reach this stage.

gradually fading out (Text Plate V., Fig. 3). The lower line of processes behaves in a slightly different manner. Instead of spreading out to form a continuous straight band it forms a series of short, nearly longitudinal bars (Text Plate V., Fig. 4). Later on these short bars fuse end to end in various fashions to form from two to five longer bands or stripes one of these extending nearly two thirds of the length of the body and the others, some above and some below the lower long stripe, vary greatly in length in different individuals (Text Plate V., Fig. 5). This process of conversion of bars into stripes takes place, as in the case of the doubling of bars in the male, in an anteroposterior direction. In half grown females all stages of the process may readily be found. Such specimens show from two to five of the posterior bars intact while the stripes at the anterior end are well marked. I have never yet seen an adult female in which all of the bars had disappeared, the most posterior one always persisting. The appearance of adult females is strikingly characteristic and must serve as a very efficient recognition mark for the males. The sexual difference is accentuated by the fact that the fins of the female are nearly or quite devoid of any dark pigment, only a small amount of a light yellowish pigment being present and that chiefly on the caudal fin.

The fins of the male bear the same characteristics as do those of *F. heteroclitus*, but are even more pronounced in their sexual dimorphism. Especially is this the case in the anal fin which is prolonged backward more markedly than in the previously described species (see Plate XXVII., Fig. 4). The anal fin of the female is swollen and inflamed in egg-laden specimens freshly brought in. The flow of milt is free and copious only in males possessing the highest coloration. The contact organs are even better developed than in *F. heteroclitus* and their distribution is very similar.

Beyond these indications I have no direct evidence bearing on the spawning behavior of *Fundulus majalis*. Although taken in large numbers when they were apparently at the height of their sexual activity, as indicated by the abundance of ripe eggs and sperm, they showed no tendency to spawn in captivity. The fish are much wilder than *F. heteroclitus* and seem to feel captivity much more keenly. All of their normal activities seem to be, for a time at least, inhibited by confinement in restricted quarters, as is indicated by the fact that they will not feed until they are nearly starving, so it is hardly to be expected that they would spawn under these conditions. I think there can be no reasonable doubt, however, that their spawning behavior closely resembles that of the species described, especially since the observed behavior of a representative of another genus, viz., *Cyprinodon variegatus*, so closely resembles that of the latter. It is highly probable, judging by the very much elongated anal fin of the male *F. majalis* that this fin plays a more important part in clasping the body of the female than does the analogous fin of *F. heteroclitus*, but the difference, like that in the other details of spawning behavior, is probably one of degree rather than one of kind.

Fundulus Diaphanus.

The following extracts, referring to sexual dimorphism, are taken from Jordan and Evermann's systematic account:

> "Fins not large; dorsal and anal rather low; ventral scarcely reaching vent in females; somewhat longer in the males. General color olivaceous; sides silvery. Male with about 20 silvery vertical bars, narrower than the dark interspaces; female with 15 to 20 transverse bars, shorter than the silvery bands of the male, the interspaces pale; back sometimes spotted; young always with black bars; fins nearly plain."

In the above description the dark coloration is arbitrarily spoken of as the background on which are superimposed silvery bands, in the case of the male. In the case of the female, on the other hand, the silvery is referred to as background for the somewhat narrower dark bars. A casual examination of the figures representing the two sexes (Plate XXVIII., 1 and 2) will show, I believe, that the dark bars are analagous in both male and female, those of the former simply being considerably broader and a little darker than those of the latter. This greater distinctness of the cross banded pattern in the males is just what we should expect to find if we compare this with the other species examined. The dimorphism in color pattern, however, has not become so pronounced in this case as in the others, and probably represents a more primitive condition.

The dimorphism in the case of the dorsal and anal fins, although not nearly so marked as in the other species, is still quite evident, especially in the case of the anal, which is considerably longer in the male. That of the female, as in the other species, is stiff and swollen, but only in the basal two thirds. The dorsal of the male is frequently colorless or nearly so, like that of the female, but is often decidedly mottled with dark pigment after the fashion seen in the male of *F. heteroclitus*, but much more lightly. The chief mottling is found on the posterior rays, a sort of prophecy of the very marked spots on this fin in other species. The contact organs are similar in form and distribution to those of *F. heteroclitus*.

In general it might be said that this species shows the beginnings of sexual dimorphism in practically all of the points that become so marked in other species, and probably represents a primitive condition.

I am sorry to be unable to present any facts regarding spawning behavior, but must plead as an excuse that this species, being a fresh or brackish water form found in a large pond on Martha's Vineyard, was inaccessible for observation in its natural haunts, and could be transported to aquaria only with the greatest difficulty. Moreover, when once they are transported and are safely housed they die off very rapidly and the diligent collector receives a poor reward for his labor. The species is not at all resistent to adverse conditions as is *F. heteroclitus* or even *F. majalis*.

The presence of a sexual dimorphism, the same in kind although different in degree, from that in other species of the same genus, lends probability to the belief that the spawing behavior is similiar to that described above.

CYPRINODON VARIEGATUS Lacépède.

(Sheephead Minnow; Purcy Minnow; Short Minnow.)

Spawning Behavior.

The opportunity was not afforded me of observing the behavior of this species in its native environment, but I was very fortunate in being able to get fairly complete records of its spawning habits by the use of aquaria.

The following observations were made on July 20:

A medium-sized aquarium was fitted up with stones *Ulva*, *Fucus*, etc., to approximate natural conditions, and five male and nine females of the species *Cyprinodon variegatus* were introduced. At about eight o'clock in the morning, when all was quiet in the room I had a fine opportunity of observing the spawning behavior of these fish. Of the five males three were in spawning plumage. one of them being noticeably more brilliant than the other two. When I first observed these fish this brightest male had acquired complete control of the situation. He was so extremely active and pugnacious that he succeeded in driving not only the other males but the females into the friendly shelter of the masses of coarse brown seaweed. Whenever any of the other fish so much as ventured to poke its head out of the shelter the ruling male would dash up and scare it into its retreat again. One of the brighter males, less subdued than the rest, ventured out more frequently and farther than the others. Instead of meekly retiring before the vigorous onslaught of the enemy, he offered considerable resistance. On several occasions the encounters between the two males developed into combats at close quarters, in which heads and jaws were the weapons. They would begin by butting heads fiercely and would occasionally grasp jaws and shake one another powerfully from side to side as though each were endeavoring to tear out his opponent's jaw. These struggles were usually short lived, one of the belligerents, invariably the one that had been in hiding, seemed to tire. They would then separate, as though by mutual consent, and the defeated male would ingloriously retreat to shelter, usually slowly as though exhausted. I have been unable to notice that either of the combatants in these frays receive any injury.

It is, I believe, more of a test of vigor and endurance than any attempt to inflict bodily injury. On the supposition, then, that sexual vigor and general bodily vigor run parallel and that the index of both is the brilliancy of coloration, we can readily understand why it is that the most brilliantly colored male is invariably the victor in these struggles for supremacy. It probably is the case too that a higher courage accompanies a higher bodily and sexual tone and makes a male at his climax practically invincible. It seemed to me, as I watched the activities of this male, that his extreme impetuosity was decidedly a detriment to him, for he defeated his own ends by driving away all females that ventured to seek the open. The ruling male always approached these females, which were ready to spawn, judging by their distended abdomens, with so much fierceness and speed that they were forced to retreat. Occasionally this male succeeded in cornering a female in one of the angles of the aquarium either at the bottom or at the sides, and spawned with her. The method of spawning is not unlike that observed in *Fundulus heteroclitus.* The male holds the female just forward of her caudal fin, using chiefly his very large, strong dorsal fin for this purpose. He lies slightly on the back of the female, but mainly side by side with her. The anal and ventral fins are used to hold the female up against the clasping dorsal, but these fins do not, so far as I was able to observe, clasp so firmly as in *Fundulus.* The approximation of sexual openings is not nearly so close as in the last-named species and hence there is probably less surety of successful fecundation. The eggs and sperm are, however extruded in such close proximity that the chances of failure are comparatively slight. While clasped by the male the female vibrated the body as described for *Fundulus* but more rapidly. In fact the whole spawning act is of such short duration that it is extremely difficult to see exactly what takes place. One has to make repeated observations, watching each detail of the process and even then some details elude ones most careful scrutiny.

July 22. — Two days later the ruling male still continued to rule although challenged frequently by the other male, now almost equally brilliant. The encounters between these two belligerents was very interesting, reminding one of nothing so much as an encounter between two game cocks. They approach threateningly, every fin erected and body quivering for the fray. Then they dash at one another, the ruling male being slightly more aggressive. After an encounter, much more evenly waged than on the former occasion, the rival male gives up temporarily and retires to his corner. He is still full of fight, however, for in a few moments he begins to make threatening demonstrations in his corner, turning sudden summersaults and making quick, active darts out and back. This behavior never fails to bring his opponent to the fray again. This sort of thing usually lasts until one of the combatants — always the lesser male — grows weary and retreats to a convenient hiding place, there to recuperate for another series of encounters. Several other fairly brilliant males could be made out, hiding in the same fashion. The females are still too frightened to emerge from shelter. The fierce aspect of these fighting males is remarkable in creatures so small. When swimming ordinarily the dorsal fin is not used, being kept folded flat on the back. But whenever fighting or sexually excited, this fin comes into action, expanding like a bat-wing sail.

The reckless courage of the ruling male surprised me. I put into the aquarium a male *Fundulus hereroclitus* of large size. Without a moment's hesitation the little warrior dashed up to the far bulkier intruder and caused the latter to beat an ignominious retreat. Several larger fish of other species met a similar reception and fate.

July 23.— On the following day the ruling male had been deposed and there was an extremely vigorous struggle for supremacy among the other males. Only one of these failed to enter the lists. This one had as yet not reached the sexual climax and was, consequently *hors du combat.* As one might have anticipated, the male that had ousted the original ruling male was the victor, the others finally acknowledging defeat by retiring to shelter. The same program was then repeated that has been described for the previous days.

The deposed male now showed signs of waning vigor in the paling of his "plumage," which was becoming rather dull at the posterior end of his body. This fading out of brilliancy proceeds in a postero-anterior direction, the top of the head being the last part of the body to lose its bright coloration.

July 29.— Both males and females of *Cyprinodon* are seen to have passed the sexual period. The females are no longer distended and no longer show any fear of the males, the two sexes mingling quite amicably. The males have all lost their spawning coloration except for traces of iridescent green about the head. The dorsal fin of the males, having lost most of its pigment, shows in one or two cases quite a noticeable dark spot on the posterior rays. This spot is, however, not nearly so distinct as that in the females. In color the males are now pale green with dusky markings that stand out somewhat more clearly now that the iridescence and general dark body coloration has nearly disappeared. The males are still greener than the females but the difference in the intensity of the coloration is far from marked.

When the fish were fed it was noticeable that the males always erected the dorsal fin when they made a dash for a fragment of food. They seem to raise this fin whenever excited in any way.

Sexual Dimorphism.

The following extracts referring to sexual dimorphism are taken from Jordan and Evermann's systematic account:

> "Body very short and robust, in adults high and much compressed, the females abruptly constricted at the base of the caudal peduncle. . . . Dorsal fin moderate, in females as high as the length of its base, in males much higher; origin of dorsal midway between base of caudal and end of snout; base of fin $1\frac{1}{3}$ to $1\frac{2}{3}$ in length of head; longest ray (in male 2 inches long) reaching half way from base of fin to base of caudal, the anterior rays equaling length of head and extending beyond tips of posterior rays when the fin is depressed; in females the longest ray about $1\frac{1}{2}$ in head; origin of anal under eighth or ninth ray of dorsal, the fin very small and much higher than long; length of base about equaling snout; longest ray half length of head (less in females). No external oviduct. Caudal truncate or slightly emarginate, $1\frac{1}{4}$ in head; ventrals, in adult males, reaching in front of anals, $2\frac{1}{3}$ in head; in females reaching vent; . . . Scales large, tuberculate in males, arranged in regular series; . . . Color: Male olivaceous; from dorsal forward above pectoral to head deep, lustrous steel blue, the color very intense and conspicuous in life; rest of upper parts with rather greenish luster, becoming dull slaty blue, and on cheeks, opercles, sides anteriorly and belly deep salmon color; lower lip and preopercle violet; dorsal blackish, the anterior margin of fin orange; caudal dusky olive with jet-black bar at tip, and a narrow black cross-streak at base; anal dusky at base, bordered entirely around with bright orange; ventrals dusky, bordered with orange; pectorals dusky orange, darker below. Smaller specimens show some orange shading on the sides, and sometimes also traces of the cross-bands of the female. Female very light olive; lower half of the sides with about 14 alternately wide and narrow vertical, dark bars, those anteriorly narrower and closer together; usually 7 or 8 dark cross-bars on the back, alternating with the wide bars below; these bars are of various degrees of distinctness, sometimes almost obsolete; a dusky area below eye; young with broad greenish cross shades wider than the interspaces; belly pale or yellowish; lower jaw largely blue; cheeks brassy; dorsal dusky, with an intense black, faintly ocellated spot near tip of last rays; caudal faintly reddish, with a black bar toward base; other fins pale orange, with some dark points. Length: Male 3 inches; female

2 inches. Cape Cod to the Rio Grande, in brackish waters, entering streams, very abundant southward, the males more highly colored southward, but the southern form (called *gibbosus*) not otherwise different."

These sexual differences, especially those of general bodily form, comparative size and shape of fins, color pattern, etc., are well brought out in the illustrations (Text Plate II., Figs. 3 and 4). Characters involving color, iridescence and the like cannot be represented in monocrome, so it will be necessary to fall back upon verbal description. For this purpose I cannot do better than to refer to Jordan and Evermann's full account for details not brought out in the illustrations. This account was evidently written with reference to the fish when in "spawning plumage," no reference being made to the fact that, in the males especially, the color and iridescence are merely temporary adornments, characteristic of the breeding season alone. Before and after the breeding season the males are about the same color as the females — perhaps a trifle greener.

The points of sexual dimorphism to be especially born in mind are the following:

1. The male is usually somewhat larger than the female — the opposite being the case in the species of *Fundulus* described.
2. The male, in "spawning plumage," is very much more brilliantly colored than the female.
3. The body of the male is decidedly deeper but more compressed than that of the female, differing from *Fundulus* in this respect.
4. The dorsal, anal and ventral fins are larger in the male than the female, even more markedly than in *Fundulus*.
5. The cross-barred color pattern is retained more nearly intact in the female than in the male, the opposite condition holding for *Fundulus*.
6. The dark, ocellated spot, that, in *Fundulus* characterizes the posterior rays of the dorsal fin of the male, is present here only in the female, although the same area in the male is usually more heavily pigmented than the rest of the fin in the male.
7. The contact organs are similar in form and distribution to those of *Fundulus*.
8. As in *Fundulus*, all of the fins of the male are more deeply and more brilliantly colored than in the female.

9. The generally aggressive and warlike aspect of the male is in striking contrast to the comparatively mild and timid aspect of the female. This contrast is more striking in *Cyprinodon* than in the species of *Fundulus* examined.

10. This exceedingly fierce aspect of the male is largely due to the very marked height of the dorsal fin and his method of carrying it spread to the utmost.

Summary and Conclusions.

Classification of Secondary Sexual Characters.

I. Of the permanent differences the following is a list:

1. Relative size of the sexes.
2. General body form.
3. Relative sizes of the various fins.
4. Shape of the various fins.
5. Color pattern (*a*) on the body, (*b*) on the fins.
6. Quality and intensity of coloration, whether it consists of pigmentation or of iridescence.
7. Relative abundance of the sexes.

II. Of the temporary differences that appear only during the spawning season the following is a list:

1. The distended abdomen of the female.
2. The swollen and inflamed anal fin in the females of *Fundulus*
3. The marked intensification of coloration and iridescence in the males, both on the body and on certain fins.
4. The paling of color in the females.
5. Contact organs in spawning males.
6. The increased activity of the males, accompanied by an increase in courage.
7. The coyness of the females, especially in *Cyprinodon.*

All of these secondary sexual characters can, I believe, be shown to be either direct adaptations to the sexual life of the fish or necessary accompaniments of the high physiological tone that accompanies the sexual climax.

Of the permanent characters the most obviously adaptive are those that have to do with the differences in size of certain fins in the two sexes. Without reasonable doubt the enlarged dorsal, anal and ventrals are adaptations to facilitate clasping. The origin of this adaptation and the way in which it is thought to have given rise to the habit of intromission will be discussed later.

The differences in shape of certain fins may, with equal certainty, be said to be adaptive. The backwardly directed prolongations seen on the anal fins of all the species of *Fundulus* examined are evidently to give the male a greater reach in his effort to clasp firmly the body of the female. The shortness and softness of the dorsal fin of the female is of advantage in that it is thus less in the way.

Differences in color pattern can only be explained as sex recognition marks. It can readily be seen, in the light of the observations on the spawning behavior of these fish, that it must be possible for the males to recognize the females at once and at a distance. Some distinctive character that would appeal to the visual sense is required. In the course of evolution those females that could readily be recognized as females would be the ones most frequently mated with and they would be the most likely to transmit this variation to their descendants. There seem to be at least two means of acquiring a distinctive appearance that would serve to mark off the females from the males. One means is to lose the common racial marking more or less completely, and thus to acquire a sort of secondary solid coloration. This method can be seen in two stages of development in two species of *Fundulus*, viz., *F. diaphanus* and *heteroclitus*. The former presents a condition in which the bars of pigment of the female are simply narrowed markedly, while the latter shows their reduction to the merest suggestion of a cross-banded pattern. The second means of acquiring a distinctive female marking is exemplified by *F. majalis*, in which the characteristic banded pattern of the species, which is possessed by the young of both species, is, during ontogeny, gradually converted into a longitudinal striping of a most pronounced order. The stages in the process of change from a transverse to a longitudinal pattern have been described in another place.

Attention has been called to the sequence of changes from the young to the adult color pattern in both male and female of *Fundulus majalis*. In the first place there seems to be a very deep-seated law of antero-posterior development. In the second place there is in the female a transition from a primitive cross-banded pattern to one characterized by longitudinal stripes. This is the opposite order of change from that given by Eimer and shows that his laws of orthogenetic variation have only a limited application. We have here a clear case of orthogenetic variation during ontogeny, a phenomenon that Gadow tried unsuccessfully to show in the case of scutes of *Chelonia*.

The total male plumage cannot be considered as primitive, the spawning plumage proper being secondary, an especial male acquisition due to his superabundant vitality. The cross-banded pattern is probably primitive and was possessed by both sexes. Even the distinct spot on the dorsal fin in the males of various species of *Fundulus* was probably a character common to both sexes, for in the allied species *Cyprinodon* we find this marking more pronounced in the female, but often present in rather vague form in the males.

Whether differences in the relative abundance of the sexes can be explained as adaptations is open to discussion. The fact that, in all the species studied, the males are relatively rare may be explained by the law of economy, for comparatively few males are quite capable of fecundating the eggs of many females, hence any more males would be superfluous. The experiment of putting too large a proportion of males in aquaria with females shows the disadvantage of a superfluity of males, for they spend most of their time fighting instead of devoting their attention to spawning.

The more slender body form of the males in *Fundulus* and the deeper but more compressed bodies of the males of *Cyprinodon* are probably both adaptions to the more active and combative disposition of that sex.

Of the temporary characters that accompany the sexual climax in both sexes, the heightened color of the males is most readily explained on purely physiological grounds. It is well known that heightened vigor, whether reproductive or somatic, is accompanied by a more active metabolism, and it is equally well known that pigmentation is a sort of index of the rate of metabolism in an organism. A heightened sexual vigor is then necessarily accompanied by an increase in pigmentation. So much for that phase of color intensification that is simply dependent on an increase of pigment; the blue gleam that appears only during active spawning must be the direct result of sexual excitement, for it fades almost immediately when the males are removed from the sexual environment. I am inclined to look upon this gleam as a sort of flush such as might suffuse the human body under excitement.

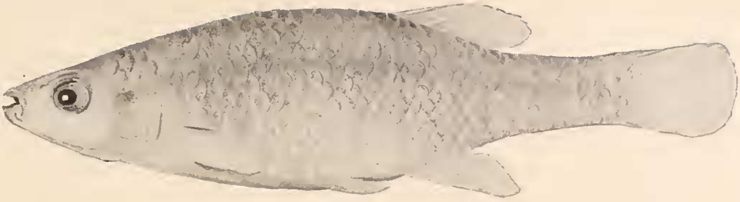
Contact organs may be considered as excrescences produced by the excess vitality of the male and specialized for an especial function. As suggested in the introduction of this paper, it is believed that both structure and function appeared in response to a heightened metabolism. After their appearance the structures were modified by use or function.

It is interesting to endeavor to trace the origin and development of the habit of intromission that seems to prevail among about half of the Pœciliidæ. This habit is invariably associated with an increase in the length of the anterior rays of the anal fin and the modification of these rays in various ways into an organ of intromission. The first step in the process was doubtless a mere elongation of the whole anal fin, as seen in *Fundulus diaphanus*. The next step was probably a more rapid growth of the anterior rays of the fin, such as we see in *Fundulus heteroclitus* and to a slightly more marked extent in *Fundulus majalis*. The function of this elongation in these species of *Fundulus* is partially to give the male a greater reach and partly to fan the sperm toward the extruded eggs. It is only a few steps farther in the same direction for the anal fin to assist the sperm to enter the oviduct of the female when internal fertilization becomes necessary.

PLATE XXVII.

FIG. 1. *Fundulus heteroclitus*, female.
FIG. 2. *Fundulus heteroclitus*, male.
FIG. 3. *Fundulus majalis*, female.
FIG. 4. *Fundulus majalis*, male.

These figures and those in Plate XXVIII. are reproduced from wash drawings and represent the actual sizes of the average adults.



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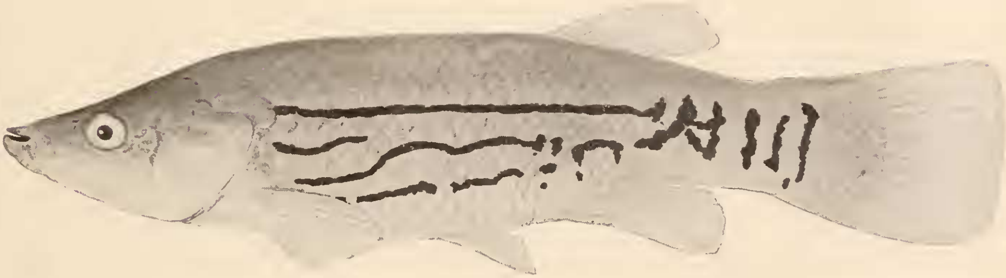

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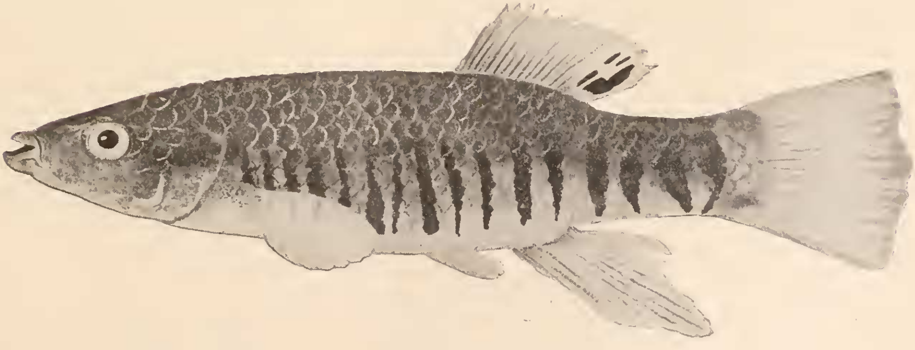

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PLATE XXVIII.

FIG. 1. *Fundulus diaphanus*, female.
FIG. 2. *Fundulus diaphanus*, male.
FIG. 3. *Cyprinodon variegatus*, female.
FIG. 4. *Cyprinodon variegatus*, male.

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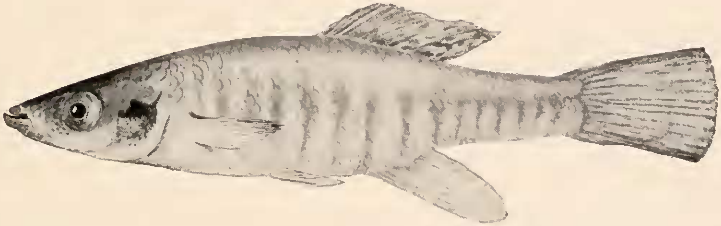

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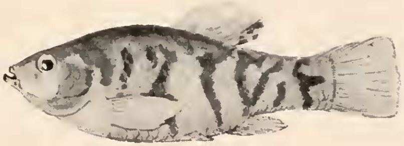

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Vol. XII. *May, 1907.* *No. 6*

BIOLOGICAL BULLETIN

THE SYSTEMATIC AFFINITIES OF THE DIPTEROUS FAMILY PHORIDÆ.

CHARLES T. BRUES.

In a paper on the nomenclature of the dipterous wing published in the current number of *Psyche*, Professor Williston ('06) has again raised the much vexed question of the relationship of the Phoridæ among the families of Diptera.

During the past few years I have devoted considerable time to a study of this small and truly remarkable group of flies, but have been unable so far to reach any wholly satisfactory conclusion as to their proper systematic position. Attempts in a number of directions have each revealed some important if not insuperable obstacle, and I had let the matter rest, compelled to accept, albeit rather unwillingly, the final decision expressed by the late Baron Osten Sacken in the following words: "A real *affinity* with *Phora* does not exist anywhere."

In the nature of things such a negative opinion on a question of phylogeny can only be tentative, and it is with pleasure that I find the discussion reopened by so eminent a dipterist as Professor Williston.

Several entomologists who have given special attention to the group have attempted to reconcile its notable peculiarities of structure with types to be found elsewhere within the order of Diptera, and one has even essayed to connect them with the Aphaniptera which are almost unanimously regarded as forming a separate order.

The antennæ and the wings are the organs which offer the difficulties and I shall describe them briefly. The antenna consists apparently of a single large globular or pear-shaped joint which bears a dorsal or terminal arista composed of three joints. When examined more closely, however, and more especially in longitudinal section, it is seen that this large segment is complex (see Fig. 1) and consists of a smaller joint almost entirely concealed within a larger outer one. These two are attached to the head by a third small and partially concealed segment. Such an arrangement is constant throughout the Phoridæ and is, so far as I know, absolutely unique among Diptera.

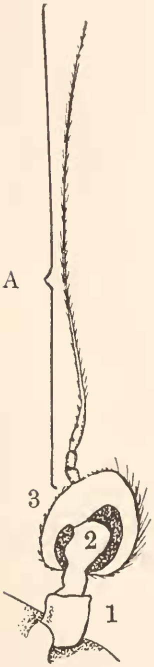

Fig. 1. Phorid Antenna. 1, 2, 3, antennal joints; *A*, arista.

The nomenclature and homology of these joints has been interpreted in several ways. Wandolleck ('98) has considered the antenna to be six-jointed without attempting to homologize the segments with those of the antennæ of other Diptera. The majority of recent dipterists seem to have accepted a nomenclature which would regard the three large basal joints as homologous with the three segments of the typical brachycerous antenna, the apical one supplied with an arista of the usual kind. The third interpretation suggested in the paper before referred to by Williston ('06) is that the basal portion consists of two joints only, and that the arista so-called really represents the whole flagellum of the nemocera. Schiner also refers to the antenna as apparently two-jointed ('64, p. 335), but on page xv he says in more detail: "Fühler nahe am Mundrande eingefügt, scheinbar zwei-gliedrig, das dritte Glied rund (bei *Conicera* kegelförmig), mit nackter oder doch nur pubescenter Rücken- oder Apicalborste." It is thus evident that he takes them to be actually three-jointed, and in this view I fully concur. From the examination of large numbers of mounted specimens representing most of the described species, as well as several in microscopical section, and a number of published figures, I am fully convinced that there are always three distinct joints, the large apical one of which bears an arista.[1]

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[1] Whether we conceive the arista to be a modified portion of the nemocerous flagellum would in this connection be a problem apart from the relationship of the various brachycerous families among themselves.

In all species that I have examined the arista is three jointed, the two basal joints short and the other long. It thus offers no noticeable difference from the same appendage of many typical Brachycera.

Williston suggests that the Orphnephilidæ have antennæ comparable to those of the Phoridæ except for the number of aristal joints which is seven. In this case however, to judge from Schiner's description[1] the second joint is composite like the third joint of the Tabanidæ, Stratiomyidæ and allied forms. Whether the Orphnephilid antenna is in reality three-jointed I am unable to say, but the annulation of the second apical joint finds no counterpart among the Phoridæ.

Williston believes that the palpi are two-jointed, and bases a part of his argument for nemoceran affinities on this supposition. From my own observation I do not believe however, that such is the case, nor can I find any reference to this effect in the literature and published figures at hand,[2] with the exception of a single diagram by Dahl ('99, p. 75, Fig. 5) where he figures the palpi of the East Indian *Phora* (*Dorniphora*) *dorhni* with two joints. I think the accuracy of this one case can be questioned as I have identified almost certainly his species among a lot of Phoridæ from New Guinea and find the palpi single jointed as usual. Becker ('01) gives careful figures of the head with palpi in a number of genera[3] but in each case there is no indication of more than a single joint. I have studied with great care the mouthparts of several of the subapterous myrmecophilous genera, but have found no trace of a second joint. Fig. 2 is reproduced from a drawing of one of these species in which there is no trace of any articulation. The palpi of the Phoridæ are always large, and are in some instances immensely swollen (*e. g.*, *Phora palposa* Zett. and *Aphiochæta magnipalpis* Aldrich ♂) but in other respects

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[1] In "Fauna Austriaca," II., 643, he says of the Orphnephilidæ: Fühler nahe am Mundrande stehend, kurz und so sich darstellend, als ob sie aus einem runden ersten, einem ovalen zweiten und einer Endborste bestünden; bei mikroscopischer Untersuchung zeigt sich, dass das zweite ovale Glied aus drei und die Endborste aus sieben walzenförmigen Gliedern besteht, deren letztes am Ende borstig ist.

[2] After this paper had gone to press, Dr. Williston called my attention to another published paper by Wesché (*Journ. R. Micr. Soc.*, 1904) in which Phorid palpi are figured as two-jointed.

[3] *Phora*, *Hypocera*, *Aphiochæta*, *Trineura* and *Metopina*.

they differ from those of the more specialized Brachycera like the Muscidea only in their more strongly developed bristles.

It is in the venation of the wings that the Phoridæ depart most strikingly from the other Diptera. This varies among the few known genera only in trivial details, so that the general type for the entire family is practically uniform. Fig. 3 shows the wing of a typical species. The veins can at once be divided into two groups, the several heavy ones which lie close together at the basal half of the wing anteriorly, and the four delicate ones which pursue an oblique course across the posterior part of the wing. This arrangement is so peculiar, that attempts to homologize the veins with those of other Diptera have proved very unsatisfactory. Several families present a somewhat similar appearance and in each case comparisons have been drawn. Williston, in the paper referred to above ('06) mentions the close similarity in venation to the Bibionid genus *Apistes*, and Becker ('01) earlier noted the resemblance to *Scatopse* another member of the Bibionidæ and to *Simulium* belonging to the Simuliidæ. It is true that there is a close superficial resemblance but no attempt has been made to homologize the veins. It is evident that *Apistes*, *Scatopse* and *Simulium* are all considerably specialized and we can indeed trace through the Bibionidæ the loss of the posterior series of veins which are quite strong in *Bibio*, weaker in *Dilophus* and still more so in *Scatopse* and *Apistes*, with a coïncident strengthening of the anterior veins and their withdrawal basally. That a resemblance between this, the result of specialization within a family of closely similar forms and another otherwise widely different family

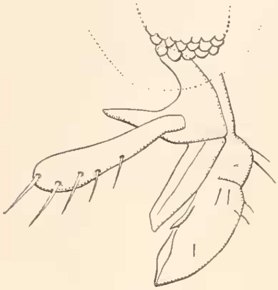

FIG. 2. *Commoptera solenopsidis* Brues, mouthparts of ♀.

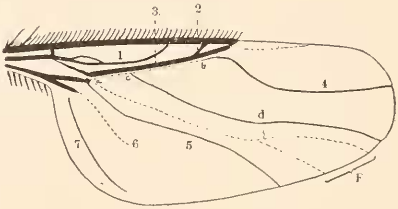

FIG. 3. Diagrammatic Phorid Wing. (After Becker.)

can have phylogenetic import, I am inclined to doubt. I think it can not be questioned that a complete venation is the primitive condition for the Bibionidæ, so that we cannot assume a common origin for the Bibionidæ and Phoridæ on evidence from neuration, but necessarily a *Scatopse*-like or *Apistes*-like ancestor for the Phoridæ. This is manifestly improbable as it involves after the fixation of a degenerating wing venation, vast strides in the evolution of other organs (antennæ, palpi) to give rise to Phoridæ while the parent stock remains unchanged.

Such a loss of posterior veins has occurred independently in many groups; the Stratiomyidæ and Hippoboscidæ may be mentioned among the Diptera and the Chalcididæ, Proctotrypidæ, Bethylidæ and Cynipidæ among Hymenoptera. The adaptation seems to be due to mechanical adjustment and of course not to common ancestry.

Palæontology offers but little on the history of the Bibionidæ and practically nothing on that of Phoridæ. The occurrence of *Phora* has been recognized by Berendt ('45) and Loew ('50) in Prussian amber. The bibionid genera *Protomyia*, *Bibio*, *Plecia*, and *Bibiopsis* have been found to occur together in considerable abundance in the Mayencian formation at Radoboj in Croatia (Heer, '47), while *Scatopse* is known first from the Ligurian, occurring in Prussian amber with *Phora* and also at Aix, France (Serres, '29).

In his monograph of the European Phoridæ, Becker has advanced the idea that the Phoridæ are derived from nemocerous forms allied to the Mycetophilidæ, basing his opinion principally upon a careful comparison of the wing venation of the two families made by Girschner. Girschner's view is given as follows: "Ich halte das Phora Geäder für ein modificiertes Mycetophilidengeäder, wie ich auch die Mycetophiliden für die nächsten Verwandten und Stammesangehörigen der Phoriden halte. Ich deute das Geäder in der oben skizzierten Weise (reproduced here as Fig. 3). Von *a* bis *b* ist die Discoidalader mit der Cubitalader verschmoltzen, wie dies schon angedeutet wird bei Macrocera. Bei einigen Phora-Formen kann man ziemlich deutlich — doch nicht so auffallend wie in der Zeichnung punctiert angegeben — die unter die Cubitalader hinstreichende Discoidalader wahrnehmen.

Die kleine Querader fehlt, weil die in der Strecke *ac* enthalten ist."

This comparison is very striking and were it not for the fundamental differences in metamorphosis and in other organs, might be conclusive. It is further strengthened by the fact adduced by Becker that certain of the macrochætæ covering the body are in both families provided with chitinous bristle-like projections.

On the other hand, Schiner has with almost equal facility reduced the Phorid wing to a more generalized type of brachycerous venation according to the following scheme (see Fig. 4). The main differences between this and the arrangement by Girschner lie in the interpretation of the fourth vein involving the presence of the cross-vein and the presence of the anal vein. Schiner's designation of the indistinct thickening along the costa beyond the tip of the heavy veins as a "Fortsetzung der ersten Längsader" I cannot exactly understand, but it involves no important point.

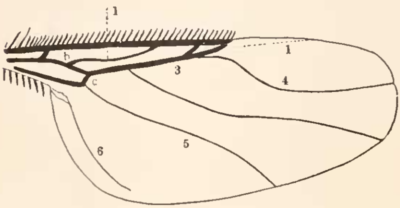

FIG. 4. Wing of *Phora* sp., as interpreted by Schiner.

Of the two views, that of Girschner accords more closely with the actual wing venation, and will I think be partly accepted with the exception of several points mentioned on a later page, by all who will study carefully a series of wings. That this similarity between the Mycetophilid and Phorid wing involves genetic relationship does not necessarily follow, however. As suggested on a previous page, specialization of wings often follows more or less parallel paths in diverse groups, and this seems to be especially true in those where a loss in complexity of structure is brought about by parasitism, secretive habits, or minute size. Turning to the Dipterous family Hippoboscidæ, widely removed from those previously discussed, we can see a strikingly similar condition to the one assumed by Girschner for the Phoridæ. Fig. 5 shows the wing of a species of *Olfersia*, a genus of Hippoboscidæ. The heavy veins are confined to the anterior basal region, while several oblique light veins traverse the large posterior expanse. Moreover, the individual veins retain almost the same relative position in these representatives of the three families. In *Olfersia* the first, second and third longitudinal veins join the heavy costal vein, which extends for only a part of the wing length, in nearly parallel directions.

FIG. 5. *Olfersia* sp., wing. *ad*, adventitious vein; *4*, fourth vein.

The fourth is not fused with the third basally, but lies close to it so that the anterior cross-vein is extremely short. Such precisely similar developments are remarkable, and must, I think, be due to some similar tendency to degenerate in certain definite directions.[1]

Be this as it may, I think the evidence is sufficient to show that direct descent from Mycetophilid-like forms is not a logical necessity in tracing a genealogy which will account for the similar wing venation of the Phoridæ.

Several other attempts to discover relationships that have been less elaborately worked out are enumerated in the papers by Osten Sacken ('02) and Coquillett ('01). There is no agreement as to whether they belong properly to the Orthorrapha or Cyclorrapha. Osten Sacken (*l. c.*) refers them to his orthorraphous superfamily Energopoda which contains the Asilidæ, Empididæ, Lonchopteridæ and Phoridæ, while Coquillett has proposed the orthorraphous superfamily Phoroidea to contain the Lonchopteridæ and Phoridæ. Mik thought he saw Borborid affinities, as he says ('98, p. 205): "Aenliche Mundtheilen . . . findet man bei den Borboriden, mit welchen die Phoriden wahrscheinlich nahe verwandt sind."

The concensus of recent opinion associates the Lonchopteridæ and Phoridæ together with apparent good reason. The head, form of front with its macrochætæ, proboscis, palpi, antennæ with their arista, bristly thorax and legs, large, freely articulated ante-

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[1] Such an idea savors of orthogenetic principles, although in an unusual sense, offering an interesting field for speculation, and for comparative studies as well.

rior coxæ, and the general habitus and actions when alive are very similar in both families. On the other hand the wing venation is different, but taken in connection with the facts already presented showing similar degeneration in so many families, this is not so great an objection as it would be otherwise.

Aside from Mik, dipterists do not appear to have detected many affinities with muscid forms. I am nevertheless inclined to agree with him that the Phoridæ show a relationship with the Borboridæ. This may quite probably also have been Schiner's idea when he placed the Phoridæ between the Bibionidæ and Borboridæ, rather than to show relationship to the former family as Williston suggests ('06). The various points of resemblance are: first, their similar appearance and actions when alive, they are the only family whose motions when running or flying could be confused with those of the Phoridæ; the modification of the incrassated hind metatarsi, the same segment being modified by rows of scaly bristles in every member of the Phoridæ, even the wingless forms and Termitoxenia; the tendency shown in the Borboridæ to lose certain of the apical and posterior parts of the veins in the wing and the tendency also to develop wingless forms like the Phoridæ. However, the legs and head of the Borboridæ are not bristly to any extent. Other differences are mainly the ones that appear equally in comparison with any family.

It is therefore in my opinion probable that the Lonchopteridæ and Phoridæ may together find a more suitable place in the Cyclorrapha rather than at the end of the Orthorrapha where placed by Coquillett. This is what Williston has done with them in the second edition of his manual ('96) where they are placed between the Platypezidæ and the Muscidea. At least there seem fewer objections to this course than to any other.

Regarding the nomenclature of the wing veins of the Phoridæ there has been considerable difference of opinion as mentioned on a previous page, and I would like to correct what I think is an error in the identification of the fourth and fifth veins. The first oblique light vein has been considered by various writers (Schiner, '62, Comstock, '94, Becker, '01, Brues, '04) as the fourth longitudinal vein, but I now believe that the second light vein represents the fourth and that what has been called the fourth is an adventitious vein. The process of coalescence in the Hippoboscidæ has suggested this.[1] Here (see Fig. 6) the first light vein arises at the cross-vein which is near the middle of the third vein. This vein is undoubtedly the fourth on account of its association with the posterior end of the cross-vein yet it occupies exactly the same place as the second light vein in the Phoridæ. This is a far more probable sort of coalescence by shortening of the cross-vein than an approximation of veins throughout their entire length which would be necessary to bring the fourth vein near the tip of the the third where the adventitious vein originates. Other points in favor of an adventitious origin are the appearance in some cases of a nebulous thickening in the same space in the Hippoboscid wing and the great variability of this vein in the Phoridæ. It also removes the difficulty which Coquillett saw in recognizing three posterior veins in the Phoridæ which he thought would exclude them from membership in the Cyclorrapha. I think also that the slight thickening near the costa beyond the tip of the third vein is also an adventitious vein if it can properly be designated as a vein.[2]

I agree with Girschner and Becker that the sixth (anal) vein is lost, or obsolete, and that the last vein (fourth light vein) represents the seventh or axillary. There can be no doubt that the short anterior branch at the tip of the third vein represents the second which is fused with it to this point. In the more specialized genera (*e. g.*, Hypocera, Puliciphora) the fusion is complete to the tip.

Public Museum Milwaukee, Wis.,
January 9, 1907.

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[1] There are many other developments among the Phoridæ which parallel those of the pupiparous Diptera such as the Hippoboscidæ. Briefly summarized they are (1) The degeneration and sometimes ultimate disappearance of the wings. (2) The production in the apterous or subapterous forms of very large eggs which distend the abdomen greatly and are even thought in some cases (cf. Wasmann,'02) to develop to the larval or pupal stage before being laid. (3) The similarity in wing venation already mentioned. (4) The degeneration of the eyes (cf. Streblidæ, Brues, 04[a], Fig. 1).

These resemblances are undoubtedly the result of parallel development, but as Professor Williston has suggested to me in a recent letter, they tend to weaken the value of the group Pupipara.

[2] Schiner refers to it as a part of the first vein.

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SOME OBSERVATIONS AND EXPERIMENTS ON THE NATURAL AND ARTIFICIAL INCUBATION OF THE EGG OF THE COMMON FOWL.

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Through a series of experiments on the developing chick, it was found that frequently the variations in development were so great that the value of the experiment was materially decreased. An attempt was therefore made to obtain more data regarding the following factors; position of eggs, turning of eggs, cooling of eggs, ventilation of eggs, moisture of eggs.

Before detailing the observations and experiments it should be emphasized that the eggs used were selected with much care. They were largely from the variety of fowls known as "Plymouth Rock." Especial care was taken to eliminate the effects of disease, inbreeding, poor food, etc. No eggs were taken from flocks in which there was not at least one cock for every ten hens. All mottled, rough shelled and ill-shaped eggs were discarded. Uniformity in size was next secured. The eggs were then tested by transmitted sunlight and those in the same stages of development selected.

Position of Eggs.

That the position of the egg during incubation has an influence on the development of the chick was shown by Dareste ('91, p. 171). It is also a well known fact among poultry raisers that the position of the egg has a profound influence upon the growth of the chick. All seem to agree that if the small end of the egg is up, the head of the chick develops in this end, and as a result, many chicks will either be deformed or fail to free themselves from the shell at the time of hatching. With these points in mind, a series of observations was made with a view of determining just what position the eggs occupy during natural incubation.

Natural Position. — Two nests containing an aggregate of twenty-seven eggs were selected which were typical of the extreme conditions. One of these was exceedingly flat, being on the ground. The other, being in a box of straw, had a very concave bottom, so that the eggs were crowded closely together; those at the periphery always rested on a sloping side.

In each of these nests, the eggs were marked so that any change of position was readily noted. A diagram of the nest was made each day, showing the position of each egg and the angle of inclination of its long axis. The sketches were made at the time the hen was feeding. If she did not leave the nest, as sometimes occurred, she was gently removed until the sketch could be made.

The angles were assigned according to the following method: an egg whose long axis formed an angle of less than 10° with the horizontal plane was marked 0°. If the long axis formed an angle of more than 10° and less than 22½° it was recorded as 15°. If the angle were more than 22½° and less than 67½° it was recorded as 45 degrees. While those whose angles were more than 67½° were recorded as 90 degrees. It was found to be impossible to register the exact angle without the expenditure of much more time than the problem merited.

In the nest with the flat bottom there was an average of less than 10 per cent. of the eggs, in which the angle of inclination exceeded 15°. From this nest of twelve eggs eleven hatched, giving a percentage of 91.7.

In the second nest which was extremely concave an average of 30 per cent. of the eggs showed an inclination of 45° or more. From this nest containing fifteen eggs, thirteen hatched, giving a percentage of 86.6. Three other nests on the ground were compared with those in straw, but in these the percentage of chicks hatched was about the same. These observations led to the conclusion that the oblique position of the egg is a factor of little or no importance in natural incubation.

It should be added that in natural incubation, one very rarely finds eggs so placed that the smaller end is uppermost. This is probably the result of two mechanical factors. Those eggs at the margin, when beneath the hen, assume an oblique position owing to the fact that this is the position in which least resistance is met; a second factor is that of specific gravity. The air space naturally tends to become uppermost and as this increases in size, the center of gravity becomes lower and lower, and the number of eggs assuming oblique positions increases as incubation progresses. It must, however, be kept in mind that an egg under natural conditions, does not maintain a position such that its angle is constant, and it may be that this varying angle of inclination influences development.

Experiments. — In order to ascertain what influence the position of the egg during artificial incubation has upon development, the following experiments were made:

In one tray of twelve-row capacity, the eggs were arranged in the following manner: Six rows were filled with eggs placed in such a position that their long axes were in a horizontal plane. Each row contained eight eggs, there being thus forty-eight eggs lying flat. In the remaining six rows the eggs were placed in such a position that their long axes coincided with an angle of 45°. Each of these rows contained ten eggs, giving sixty eggs placed obliquely. That the value of the results might not be lessened through the introduction of bad lots of eggs, they were controlled by taking equal numbers of these eggs from three different flocks of fowls and evenly distributing them throughout the tray.

On the fifth day thirteen infertile eggs were removed from those placed obliquely, leaving forty-seven living embryos. These eggs were again examined on the sixteenth day, and five dead embryos were found, leaving forty-two chicks alive at this time. Forty chicks hatched; the remaining two died after having pipped; the percentage of hatched chicks from the fertile eggs being 85.0.

From the forty-eight eggs lying flat, ten infertile eggs were removed on the fifth day, leaving thirty-eight live embryos. The sixteenth day, nine dead embryos were removed, leaving twenty-nine living chicks; of these eighteen hatched; three died after having pipped, and the remainder died in the shell. . The percentage of chicks hatched from the fertile eggs was 47.3.

It is, of course, perfectly obvious that in this experiment, the results indicated that eggs placed obliquely hatched a far higher percentage than those placed flat.

In a second experiment, the eggs were arranged in precisely the same manner, but in another incubator provided with a special ventilating apparatus. The fifth day, the eggs placed obliquely were tested and eight discarded. On the ninth day, three dead embryos were removed, and on the sixteenth day, four more were dead, leaving a total of fifty-three living eggs; forty-seven chicks hatched. Of the remaining six, four were dead in the shell, and two pipped. Thus there were hatched from those eggs placed obliquely, 88.7 per cent. of the fertile eggs.

From the forty-eight eggs placed flat, seven were found infertile on the fifth day. On the ninth day, six dead embryos were removed. On the sixteenth day, but one dead embryo was found. Thirty four chicks hatched. There was hatched about 83 per cent. of the fertile eggs.

The results of the foregoing observations and experiments taken together, lead to the deduction that when the supply of fresh air (oxygen) is inadequate, the oblique position of the egg, thereby bringing the embryo in closer contact with the air chamber, is decidedly advantageous. When there is an abundant supply of fresh air, there is but little to be gained through placing the eggs obliquely.

Turning of Eggs.

How many times the hen turns her eggs during the time of natural incubation is a question often asked, but as yet unanswered. Reaumer ('49, p. 166) states that they are turned daily and Dareste ('91, p. 161) that they are often turned twice daily. Believing this to be a point well worthy of investigation, a series of observations was made with the hope of obtaining some information as to the influence this factor plays in development.

Experiments have previously been made with a view of ascertaining what influence turning the egg has upon development. Dareste ('91, p. 165) placed sixteen eggs under the same conditions of artificial incubation. Eight were unmoved, while the remaining eight were turned twice each day. In the first set absorption of the yolk did not occur in any case; the embryos died during the second or third week. Of the eight which were turned six developed normally ; a seventh was opened on the twenty-second day, showing normal conditions ; while in the eighth the chick died on the twentieth day, the adhesions between the allantois and the yolk having prevented the absorption of the latter.

Natural Turning. — In order to obtain satisfactory data, it was necessary to arrange nests in such a manner that the position of each egg could be sketched at frequent intervals without disturbing the hen. Three nests with felt sides and concave glass bottoms were constructed, and placed in such positions that the eggs could be viewed and sketched from below. The eggs were numbered in four places about equally distant from each other and midway between the two ends of the egg. Each figure bore an alphabetical index. The numbers on egg 1 would run thus : 1*a*, 1*b*, 1*c*, 1*d*, so that any degree of turning might be readily observed. Five sketches or plats of each nest were made each day during the incubating period, and at the following hours : 6 A. M., 9 A. M., 12 M., 3 P. M., 6 P. M.

The observations show that the eggs are turned partially or completely much more frequently than has been supposed, at least five times during any given day. It should also be stated that on a number of days sketches were made at more frequent intervals, and in nearly every case the eggs had been partially or completely rotated. The observations made were confined to the twelve hours between 6 A. M. and 6 P. M. A number of scattered observations lead to the belief that the eggs are also turned during the night, but just how frequently is yet to be determined.

The hen turns the eggs in two ways. If a sitting hen be watched as she returns from feeding to sitting, it will be seen that she moves her body rapidly from side to side. Whether the object be to turn the eggs is uncertain. Probably the first object is to bring the surface of the body in the closest possible contact with the growing embryos. Accidentally or purposely, she also turns the eggs. This is not only true of the hen returning from feeding, but also when on the nest, for she is frequently observed moving about and settling down with the same characteristic lateral movements. Sometimes there are so many eggs in the nest or they are so widely scattered that the hen fails to properly cover them. When such conditions occur, the hen invariably uses her beak to bring the outlying eggs in contact with her body. Not only does she frequently thus turn the eggs, but also she very often reaches beneath her body and turns the eggs lying near the center of the nest. Why she does this is a question which awaits an answer.

Experiments. — Seventy-five eggs were selected from three lots of fowls, arranged in three groups and so placed that the eggs from one lot of fowls alternated with those from another lot. Those eggs of group I. were left unmoved ; those of group II. were turned at 6 A. M., and 6 P. M.; those of group III. were turned at 6 A. M., 9 A. M., 12 M., 3 P. M. and 6 P. M.

From group I., five infertile eggs were removed on the fifth day, together with eight dead embryos, six of which had grown fast to the shell membrane. The eggs were again examined on the twelfth day and five more dead embryos were removed. These were examined and four were found to have the allantois grown fast to the yolk ; but three chicks hatched from the entire twenty-five. The remaining four were dead in the shell. The number hatched was 15 per cent. of the fertile eggs.

In group II. there were three infertile eggs removed on the fifth day, and one dead embryo which had adhered to the shell membrane. On the twelfth day four dead embryos were removed, one had the allantois adhering to the yoke. In the others the cause of death could only be surmised. From the remainder, ten chicks were hatched ; the others died in the shell, giving a hatch of 45.4 per cent. of the fertile eggs.

In group III. six infertile eggs were found on the fifth day and no dead embryos. On the twelfth day one dead embryo was found. Eleven chicks hatched ; two died after having pipped, while the remaining five were dead in the shell ; the number of hatched chicks being 58 per cent. of the fertile eggs. The experiments indicate that frequent turnings (at least five) give best results.

It is necessary to point out, however, that a very low percentage of chicks hatched even in group III. This is to be attributed directly to a lack of sufficient oxygen, the incubator in which the experiments were made being poorly ventilated, owing to the fact that the ventilating system had been modified in order to compare its results with those provided with special ventilation. A second incubator provided with a special ventilating apparatus, hatched 83 per cent. and 88.6 per cent. of two lots of eggs taken from the same fowls.

Dareste concluded from his experiments that during the first week of artificial incubation, eggs which are unturned develop in essentially the same manner as those which are turned. The principal cause of death is due to the allantois growing fast to the yolk, causing the rupture of the vitelline membrane, thereby allowing the yolk to escape so that it cannot be taken into the body of the embryo. Dareste adds that when the eggs are turned it is probable that the position of the allantois is shifted, and this movement prevents its adhesion to the yolk. It should be remarked that during the early days of incubation it is also necessary to turn the eggs frequently; otherwise, the embryo grows fast to the shell membrane. This has not only been shown by the preceding experiments, but has been repeatedly observed in other eggs.

IV. Temperature of Eggs.

Repeated attempts have been made to ascertain the temperature of the egg during natural incubation, but as yet the results are far from satisfactory. This is due to the difficulty experienced in testing the temperature of different parts of the egg. The fact that the egg comes in contact with a heating surface above and a cooling surface below, leads to most perplexing complications. While the temperature of the hen is easily ascertained, it is not an easy matter to know the precise degree of heat applied to the surface of the egg. Moreover, it should be kept in mind that during incubation, not all the eggs are at all times in contact with the body of the hen. A layer of feathers intervenes to modify the temperature, this layer varying greatly in thickness in different parts of the body, and at different times during incubation. All these factors conspire to make an exceedingly difficult problem.

Natural Temperature of Hen. — In attempting to determine the daily temperature of the hen, special self-registering thermometers were fastened to blocks so cut that their upper surfaces were nearly egg-shaped. The lower surfaces of the blocks were broad and flat, so that they could not be easily overturned. One was placed in each of four nests and left for two or three hours, when the reading was made.

The following table shows the temperatures obtained by this method, during twenty days of incubation. In this as in subsequent tables, the Roman numerals indicate the serial numbers by which the hens were designated, while those above the columns indicate the day of incubation.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
I.	102.1	103.0	103.0	103.8	105.0	104.5	105.0	105.0	106.2	106.0	105.0	104.5	105.0	105.5	104.5	105.5	104.8	105.0	104.5	105.5
II.	103.0	104.0	103.5	104.5	104.5	104.0	105.0	105.5	104.5	104.6	104.6	104.5	104.6	104.2	105.0	104.8	105.0	105.0	105.0	104.0
III.	102.0	102.0	103.0	103.0	105.0	105.0	104.5	104.0	104.5	104.0	104.0	105.0	104.0	103.6	104.0	105.2	104.2	103.5	103.0	104.0
IV.	101.5	102.5	102.5	103.0	103.5	104.0	104.5	104.5	105.0	105.0	104.8	105.0	104.5	105.0	104.8	105.0	105.0	104.5	104.5	105.0

A second series of readings was made by gently removing the hen from the nest and placing the thermometer in the groin for five minutes. The results are of course, somewhat unsatisfactory, since the excitement of the fowls due to their being removed from the nest, results in a temperature somewhat higher than the normal.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
I.	103.0	104.0	103.5	104.6	105.5	105.0	106.0	106.0	105.5	106.0	106.5	105.8	105.6	105.5	106.2	106.2	106.0	105.5	105.8	105.5
II.	104.0	105.2	105.5	105.5	105.5	106.5	105.2	106.0	106.0	107.0	106.2	106.5	106.5	106.0	107.4	106.0	106.0	106.5	106.2	106.5
III.	103.5	103.2	105.5	106.5	106.2	105.0	105.2	105.0	105.0	105.0	105.8	105.0	105.2	106.2	106.5	106.0	106.5	106.0	106.2	106.0
IV.	102.0	102.5	104.0	104.5	105.0	105.0	104.5	105.0	105.0	105.5	105.0	105.2	105.6	106.0	105.5	105.2	105.0	105.0	104.8	105.0

Natural Temperature of Egg. — Since experiments show that the above temperatures are too high for artificial incubation, it is necessary to push the inquiry a step further with a view of determining the exact temperature of the egg during natural incubation. The temperature of the hen recorded below was obtained from a thermometer attached to a block as described above. The temperature of the egg was taken in the following manner: A pail of lukewarm water was brought to a temperature of 98° F. (by the addition of warm or cold water). The egg was then placed in a tightly fitted rubber bag and held about four inches below the surface of the water. An opening was then made in the shell directly over the embryo, and a self registering thermometer warmed to 98° inserted for five minutes. The thermometer was inserted just far enough to bring its lower end at the center of the egg. As often as the eggs were broken for testing, they were replaced by eggs taken from other hens set at the same time.

	1	2	3	4	5	6	7	8	9	10
Hen.	102.2	103.0	103.5	104.0	103.8	105.0	104.6	104.5	105.0	105.0
Egg.	98.0	100.2	100.5	100.5	100.4	101.0	101.8	102.5	101.6	102.0
	11	12	13	14	15	16	17	18	19	20
Hen.	104.8	105.2	104.5	105.0	105.2	105.0	104.6	104.8	104.5	104.5
Egg.	101.8	102.2	102.0	102.5	102.0	103.0	102.4	103.0	103.0	103.0

A second series of readings was made by gently removing the hen from the nest and placing the thermometer in the groin for five minutes. The results are of course somewhat unsatisfactory since again the excitement of the fowls, due to their being removed from the nests, resulted in temperatures somewhat higher than normal.

	1	2	3	4	5	6	7	8	9	10
Hen.	103.0	105.0	104.8	104.2	105.2	105.0	104.8	104.8	105.0	105.0
Egg.	99.5	100.0	100.2	100.5	100.6	101.0	100.5	100.5	101.5	101.5
	11	12	13	14	15	16	17	18	19	20
Hen.	104.8	105.0	104.8	104.8	105.2	105.0	105.5	104.0	104.0	104.0
Egg.	101.5	101.2	100.8	101.8	102.0	101.8	102.2	102.0	102.4	102.4

While the above are the only sets of daily observations, they were supplemented by a number of scattered tests. In no case was the egg found to exceed the temperature given in the table by more than one degree. In but few cases was it found to be a degree lower. Although it cannot positively be stated that these tested eggs would have hatched, the inference seems more than probable, since in five other cases where hens were set on eggs from the same flocks of fowls, the fertile eggs hatched with but very few exceptions. These observations show that the proper incubating temperature of the egg is about 100° for the first week; 101° for the second, and 102°–103° for the final week.

Temperature of Artificially Incubated Eggs. — The next problem is to determine what temperature must be kept in the air chamber of the incubator in order to obtain the above temperature of the egg. A series of observations was made on the artificially incubated egg. The temperature of the egg chamber was read from a thermometer placed flat and on a level with the top of the eggs, but not in contact with them. The temperatures of the eggs were taken in precisely the same manner as in the preceding experiment.

	1	2	3	4	5	6	7	8	9	10
Inc'b.	103.0	103.5	103.0	104.0	103.5	104.0	103.0	103.5	105.0	103.0
Egg.	100.2	100.0	100.0	101.2	101.6	101.8	101.6	100.0	102.8	101.0

	11	12	13	14	15	16	17	18	19	20
Inc'b.	103.0	104.0	105.0	103.0	104.5	103.0	105.0	104.5	106.0	106.0
Egg.	102.0	102.6	103.7	102.5	103.6	104.8	104.0	104.0	—	—

The above record was made from an incubator which hatched about 85 per cent. of the fertile eggs. The hatch, however, was somewhat premature, since many of the eggs hatched on the nineteenth day. It is thus evident that a temperature somewhat too high had been carried. In view of the irregularities of the incubator a second experiment was made.

	1	2	3	4	5	6	7	8	9	10
Inc'b.	102.0	102.0	103.0	102.0	102.5	103.0	102.5	102.0	103.0	103.5
Egg.	99.5	100.0	101.0	100.5	100.5	101.0	100.0	100.0	101.0	101.5

	11	12	13	14	15	16	17	18	19	20
Inc'b.	103.0	103.5	104.0	103.5	104.0	104.5	104.0	103.5	104.0	104.5
Egg.	101.5	101.8	102.0	102.5	103.0	103.0	103.0	102.5	102.5	103.5

As will be observed, the incubator was under better control in the second experiment and the chicks hatched on the twentieth and twenty-first days yet the percentage hatched was about the same as in the first, not including in either case the eggs destroyed in making the tests. While a more extended series of tests would be highly desirable, one certainly does not widely err in stating that the most favorable temperature within the egg chamber is close to 102°–103° F. the first half of the incubating period and 103°–104° F. for the latter half.

In any consideration of temperature, the fact must be kept in mind that as the chick grows, it gives off more and more heat, so that if an incubator of 200 egg capacity were entirely without artificial heating, the temperature would be much higher than that of the surrounding atmosphere; it consequently follows that less artificial heat is necessary during the later stages of incubation. The 102°–103° in the earlier stages is largely artificial heat, while the 103°–104° in the later stages would be the combined animal heat, given off by the egg, and the artificial heat supplied by the heat radiator.

V. Cooling of Eggs.

How frequently the eggs should be cooled and for how long a period, is a question of considerable importance. Not being entirely satisfied with the data at hand, an attempt was made to gather some information by actually watching a number of hens from day to day.

Natural Exposure. — Six hens were observed throughout the period of incubation and the results tabulated. It was found that the average time the hen leaves her nest, during the first fifteen or eighteen days is about thirty minutes. During the last few days of the incubating period she rarely leaves the nest. The longest time a nest was left exposed was an hour and twenty minutes, and the shortest time about twelve minutes. If the hen be obliged to forage for food, she remains a much longer time than when food is at hand. But one or two instances were noted in which the eggs were exposed for much more than an hour. It may be stated with a fair degree of certainty that the cooling of the eggs is due to the necessity of obtaining food, and in no way fundamentally affects the growth of the chick when there is an abundant supply of fresh air. There is not the least doubt, however, but what it has a beneficial influence in cases of poor ventilation, and since no incubator is supplied with too much, it probably is best to adopt the common practice of cooling the eggs. In so doing it would not seem advisable to cool the eggs for more than twenty to thirty minutes each day, for the first fifteen or eighteen days.

VI. Ventilation of Eggs.

Natural Ventilation. — In natural incubation a perfect ventilation exists. An abundance of fresh air can always reach the eggs by diffusing through the feathers which cover them. This process is constantly going on during incubation, the foul air likewise having free exit. There is thus ample opportunity for a continuous circulation of air, and there is every reason to believe that it takes place. There is also afforded by the feathers a complete barrier against sudden draughts of air. The fresh air is also raised to a certain temperature through the heat of the hen before it comes in contact with the eggs, which also serves to reduce any excessive humidity. This perfect system of ventilation cannot fail to impress one of its importance in facilitating the growth of the chick.

Dareste ('91, p. 150) conducted the following series of experiments: All the apertures of the incubator were closed during incubation, and upon examination it was found that nearly all the embryos had died. It was found further, that there had developed in the albumen a microscopic organism resembling the ordinary yeast plant. The author concludes that air modified by embryonic respiration, facilitates the growth of parasitic organisms.

Gerlach ('82, p. 115) found that by diminishing the quantity of air during incubation, he could cause dwarfing of the embryo. He then tried whether an increase in the size of the embryo could be brought about by increasing the quantity of air. A part of the shell was scraped very thin and placed in an incubator. During the first two days the normal and modified eggs were alike, but after that time the embryo in the scraped eggs developed at a remarkably rapid rate, nearly twice as fast as in normal growth.

A second method of increasing the supply of air was to remove whole pieces of the shell. Of course great care was taken not to injure the shell membrane or growing blastoderm. This fracture was made some distance from the embryo, so that the drying could not extend to the embryo, and the egg after the removal of the part of the shell was turned so that the broken portion was downward. The embryo was perfectly formed, but grew at the same astonishingly rapid rate.

The above consideration led to the conviction that artificial incubation can only proceed where there is an abundant supply of fresh air (oxygen). In order to confirm this supposition, the following experiment was tried.

Experiments. — Two incubators with similar ventilating systems, which, however, were believed to be inadequate, were employed. One was left with the ventilating system unmodified. The other was provided with two one-inch intake pipes. These extended to the outside of the building in which the incubators were located, and so arranged that a continuous current of fresh air passed into the egg-chamber.

Two egg trays of 100 capacity each, were filled with eggs from the same lots of fowls ; special care being taken to divide the eggs from each flock so that there should be an equal number in each tray. The eggs were then subjected to exactly the same treatment, barring slight variations in temperature which necessarily existed.

On the fifth day the eggs were tested, and from the incubator with special ventilation, sixteen (infertile) eggs were removed. From the other, twelve (infertile) eggs were removed. On the twelfth day they were again examined. From the incubator with special ventilation seven dead embryos were removed and from the other, twenty. From the eighty-four in the incubator with special ventilation, seventy-two hatched, while five were dead in the shell, giving a percentage of 85.7 per cent. hatched from the fertile eggs. Of the eighty-eight eggs remaining in the other incubator, but thirty-nine hatched ; a number of the remainder pipped, just how many was not recorded, while a large number were dead in the shell. There was thus hatched in the incubator without ventilation 44.3 per cent. of the fertile eggs.

But when a perfect ventilation has been obtained, it has produced certain deleterious effects which must be corrected. It is commonplace to say that when evaportion goes on in still air, this air soon becomes saturated, and evaporation, if not stopped, goes on very slowly. If, however, the saturated air is constantly removed and dry air takes its place, the rate of evaporation is increased. It is thus evident that any discussion of ventilation must take into consideration the question of moisture.

VII. Moisture of Eggs.

There is probably no one factor so little understood as that of moisture. The most careful observations of the nesting habits of the hen seem to only complicate the matter. A hen may build her nest on the ground, or in the hay loft, and in each case hatch about the same percentage. These facts, which are a matter of every-day observation, lead us to believe that eggs hatch equally well under these variable conditions. The moisture necessary for development must then be controlled by the hen, or egg, or both.

There are certain constant factors in the production of moisture which we may accept as existing. First of all, the temperature of the air in the nest is far higher than that of the outside air. As the two come in contact, there is more or less moisture produced. A second source is from the perspiration of the skin. A third source is from the egg itself. These three sources supply, so far as we are able to determine, the moisture necessary for the normal development of the egg.

It is known through the experiment of Reaumer, that excessive moisture gives rise to the pathological forms. Dareste (p. 159) also records an experiment in which the atmosphere was saturated and as a result the albumen liquified and leaked through the shell. Furthermore, Dareste, stated that excessive moisture facilitates the growth of parasitic forms which develop in the albumen.

The writer made a series of daily hygrometer tests with a view of ascertaining just how much moisture existed in the nests. In testing with the hygrometer, it was placed in the nest among the eggs, and at the end of fifteen minutes was taken out and the reading recorded. Although a great number of these tests were made and tabulated they were later discarded, owing to wide variations in the hygrometers and the problem attacked in another way. It is of course well known that the egg decreases in weight during incubation and that this is due chiefly to the evaporation. In order to find out definitely how much evaporation goes on during natural incubation, thirty-six eggs were weighed each day for twenty days and these weighings tabulated. It was found on the average that the egg during natural incubation loses about 13 per cent. of its original weight.

It was also found by experiment that the evaporation could be lessened until the egg lost but 9 per cent of its original weight and still give a healthy chick. It was likewise learned that evaporation could be increased up to about 20 per cent and the eggs give rise to perfect chicks. It would thus appear that the moisture in the incubator should be so controlled that it will allow the evaporation of about 13 per cent of the original weight of the egg.

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A PECULIAR PELVIC ATTACHMENT IN NECTURUS MACULATUS.

CLARA HEPBURN.

While studying the vertebral column of *Necturus maculatus*, in the regular class work, I found in my specimens a peculiar form of pelvic attachment which seems remarkable enough to mention.

The pelvis was attached to the eighteenth vertebra and, in addition, there were two rudimentary ribs on the nineteenth. There was nothing strange about the twentieth but on the twenty-first there was one half of a hæmal spine, that of the right side, the first complete hæmal arch being on the twenty-second.

For comparison in order to show its anomalous character, it is necessary to give the statistics concerning the conditions as reported hitherto. F. Smith, '00, and Wilder, '02, have summarized the results hitherto reported, including the papers of Parker, '96, Bumpus, '97, and Waite, '97.

In one hundred and fourteen specimens tabulated by Smith, the sacral vertebra was the nineteenth in eighty-one cases, the twentieth in sixteen and the twenty-first in one, this latter being the most posterior position ever recorded. In twelve cases the pelvis was oblique and of these three made use of the eighteenth on one side. In four cases there were three sacral ribs, that is, two upon one side and in one of these, figured by Smith on page 638, the ribs involved were the eighteenth on the right and the eighteenth and the nineteenth on the left. Thus in Smith's one hundred and fourteen cases, four involved the eighteenth vertebra but all were asymmetrical. The one which approached nearest my specimen is the one figured by Smith, where there was a ligamentous connection between the rib rudiment on the nineteenth and the sacrum, and as my specimen had been macerated before I examined it, I cannot tell whether there was such a connection or not.

As Wilder has used the same authors for his summary as has Smith, it is unnecessary to give his report.

None of these authors mention a case with the symmetrical pelvic attachment of both sides upon the eighteenth vertebra, nor one with symmetrical rudimentary ribs on any vertebra posterior to the sacral connection. It is rather significant that the nineteenth vertebra, the one that in the majority of cases bears the sacral ribs, should, in my specimen, have borne the rudimentary ones.

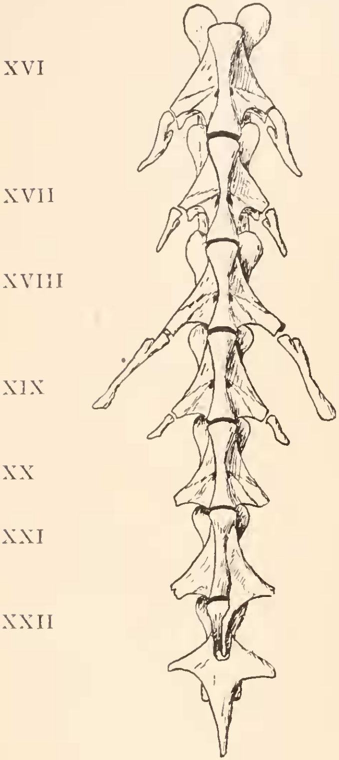

Fig. 1.

The fact that in this particular case, the first entire hæmal arch was found on the twenty-second vertebra, is not so remarkable. F. Smith records this position in sixty-two cases out of two hundred and forty-one. Wilder, also, states that the hæmal arch appears suddenly on the twenty-second to the twenty-fourth vertebra, usually the twenty-third. He then adds that in one case the vertebra just anterior to the first one bearing a complete hæmal arch, bore upon one side a slender process, four to five millimeters long, evidently representing an incomplete hæmal arch. But whether the vertebra which bore the complete hæmal arch was the twenty-second, twenty-third or twenty-fourth he does not say, so that one cannot tell whether the vertebra with the incomplete hæmal arch was the twenty-first, as in my specimen, or one of the two next posterior.

Although Bumpus has shown by a careful comparison that the variation of the position of the first hæmal arch is entirely independent of that of the sacral vertebra yet the presence of even a partial arch upon a vertebra anterior to any previously recorded, when taken in connection with the extreme anterior location of the sacral connection, appears important.

Summarizing, then, the points of especial interest in the case in question, we have the following:

1. The pelvic attachment to the eighteenth vertebra on both sides.

2. The presence of a pair of rudimentary ribs upon the nineteenth vertebra, the position of the usual pelvic attachment.

3. The presence of an incomplete hæmal arch upon the twenty-first vertebra.

Smith College,
December 11, 1906.

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NOTE ON THE CONUS ARTERIOSUS OF MEGALOPS CYPRINOIDES (BROUSSONET).

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Since describing the conus arteriosus in *Tarpon Atlanticus*[1] I have been fortunate in securing a specimen of *Magalops cyprinoides.* For this I take the present opportunity of thanking Professor David Starr Jordan.

The fish in question, preserved in alcohol, measures 19 cm. (including caudal fin) so that the heart is extremely small, and is, on account of its somewhat friable condition, difficult to handle.

The conus is everywhere quite obvious from the exterior. Fig. 1, drawn from the left side, indicates that the general form of conus and bulbus resembles that of *Amia* rather than that of *Tarpon.*

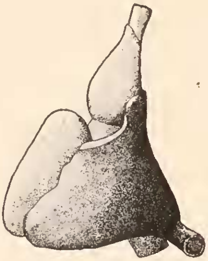

FIG. 1.

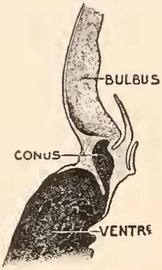

FIG. 2.

FIG. 1. Entire heart of *Megalops cyprinoides* from the left side, × 3. A small portion of the atrium has been removed to display the conus more fully.

FIG. 2. Frontal section through the right side of the heart of *Megalops cyprinoides*, showing the relations in the conus region, × 10.

The heart was opened by a mid-ventral sagittal incision, it having been previously ascertained that such an incision would fall between the valves without cutting them. After examination and

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[1] BIOL. BULL., February, 1907, p. 145. (The literature on the conus arteriosus is given in this article.)

measurement the two halves were imbedded in celloidin and cut into serial sections.

The extreme length of conus is 1 mm. in the mid-ventral line and 1.5 mm. in the mid-dorsal and lateral lines. The ventricle, from apex to junction with conus, has a mean measurement of 5.5 mm. The proportion in mean length of conus to ventricle is therefore 1 to 4.

The conus contains two transverse tiers of valves, each tier consisting of a right and left cusp placed symmetrically with regard to the mid-sagittal plane. The general arrangement agrees closely with that found in *Tarpon*, but the proximal cusps appear to be proportionately more capacious.

The conus in *Megalops* not only projects more freely from the ventricle than in *Tarpon*, but is of greater proportionate length. It would seem to resemble more closely the conus of *Albula* (as described by Boas, '80) except in the absence of the subsidiary valve cusps of the latter.

It should be noticed that the heart described is from a young fish, also that the measurements are, at best, approximate; therefore, comparisons with adults of other genera, if pushed too closely, are apt to be misleading.

The atrio-ventricular valve has three cusps.

A specimen of *Chanos chanos* (Forskål), for which I also have to thank Professor Jordan, presents an easily recognizable vestigial conus arteriosus, but only one tier of valves.

St. Louis, Mo., February 1, 1907.

SEX DIFFERENTIATION IN LARVAL INSECTS.

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The question of the causes of sex differentiation is a problem to the solution of which we seem now to be only a little nearer, despite numerous researches, than we were many years ago. It is advisable, perhaps, to continue to attempt to overcome some of the outworks of this well entrenched problem. One of the outliers of the main problem may be described in the phrase "When is sex differentiated?", another in the phrase "Does nutrition affect sex?"

Being engaged in rearing experimentally large numbers of silkworm moths, *Bombyx mori*, I have taken advantage of the opportunity to test for this species both these subsidiary parts of the sex differentiation problem. Various lots of larvæ were set apart, each individual being isolated so as to insure identity of nutrition conditions, and fed on short rations. The result of these experiments is given in a paper in the *Journal of Experimental Zoology* (vol. I, pp. 357–360, 1904). It is sufficient

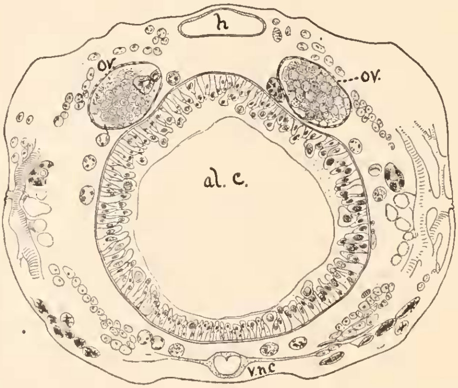

FIG. 1. Section through female *Bombyx* larva, just after the third moult; *h*, heart; *al. c*, alimentary canal; *v. n. c*, ventral nerve cord.

to say here, in a word, that this "short feeding" produced no apparent effect in determining the sex of the moths.

Since these experiments I have learned a good reason why the nutrition of the larva, at least after its first moulting, should have no effect on the sex as revealed in the moth, and this reason is that the sex of each individual is definitively determined at least as early as immediately after the first larval moulting, as may be readily perceived by an examination of the rudimentary reproductive glands.

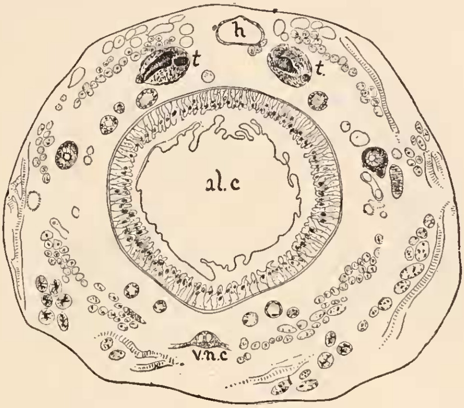

FIG. 2. Section through male *Bombyx* larva, just before the third moult; *h*, heart; *al. c*, alimentary canal; *v. n. c*, ventral nerve cord; *t*, *t*, testes.

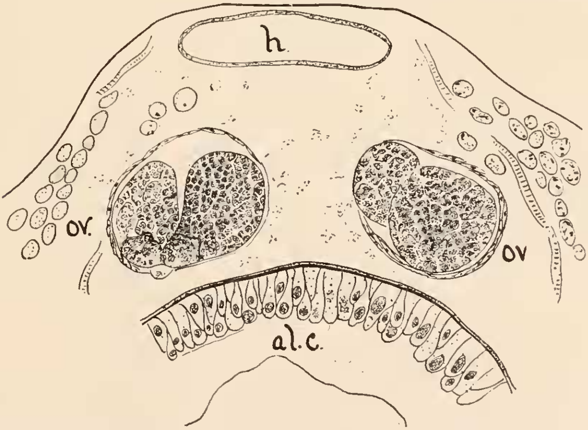

FIG. 3. Section (dorsal third) through female *Bombyx* larva, just after the second moult; *h*, heart; *al. c*, alimentary canal; *ov*, *ov*, ovaries.

In the larval silkworm there may be noted on the dorsal wall of the fifth abdominal segment two low tubercles, rather dark colored. Directly beneath these spots lie the developing reproductive organs (ovaries, testes). By dissecting a number of larvæ in their last or next to last intermoulting period, a marked difference will be noted in the size of the organs in different individuals. By dissecting out the organs and sectioning them, constant marked histologic differences will be seen in the two sizes of organs. As a matter of fact the larger are ovaries, with well-developed rudiments of egg-tubes, the larvæ possessing

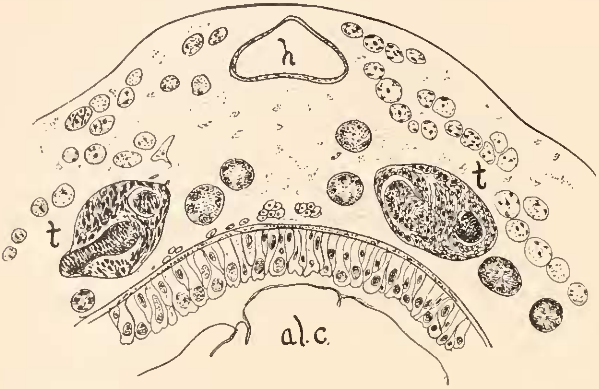

FIG. 4. Section (dorsal third) through male *Bombyx* larva, just after the second moult; *h*, heart; *al. c*, alimentary canal; *t*, *t*, testes.

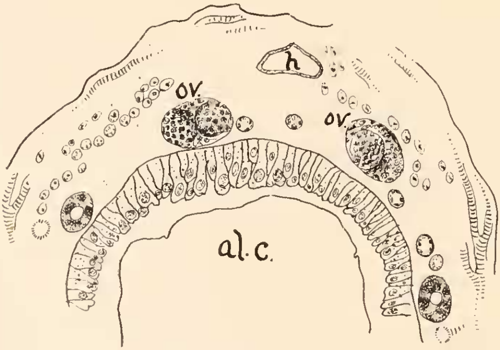

FIG. 5. Section (dorsal half) through female *Bombyx* larva, just after the first moult; *h*, heart; *al. c*, alimentary canal; *ov*, *ov*, ovaries.

them being female individuals, while the smaller are testes. By dissecting larvæ of successively younger age, these differences in size and histologic character may be followed back to just after the first moulting.

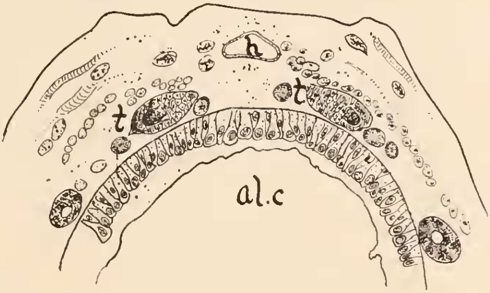

FIG. 6. Section (dorsal half) through male *Bombyx* larva, just after the first moult; *h*, heart; *al. c*, alimentary canal; *t*, *t*, testes.

Figs. 1 to 8 show, with little need of explanatory description, the gross character of the histologic differences, the relative sizes (in cross-section) of the two kinds of organs, and also the relative size of these organs in the various larval stages. I have not been sure of being able to distinguish between the sizes or the histologic characters of the reproductive organs in the just

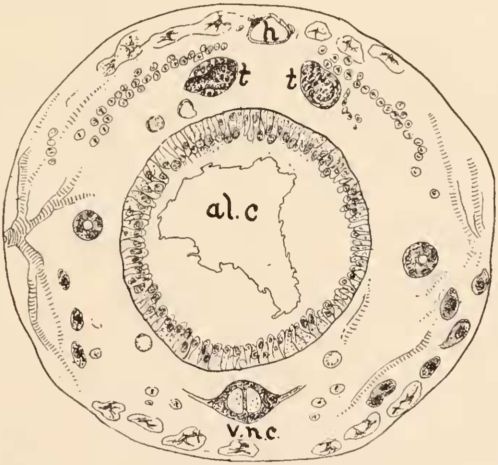

FIG. 7. Section through male *Bombyx* larva, just before the first moult; *h*, heart; *al. c*, alimentary canal; *v. n. c*, ventral nerve cord; *t*, *t*, testes.

hatched larvæ, but even here the organs are conspicuous and well started in development, being larger in diameter than the heart or the spinning glands.

We may affirm then positively that from the time of the first moulting on the silkworm larva has its sex determined: its reproductive glands are ovaries or testes distinguishable by obvious histologic characteristics. Also that the just hatched larva has reproductive organs already well developed. I have little doubt that careful scrutiny of the organs at this stage would reveal to the trained histologist, especially to the student of oögenesis and spermatogenesis, definitive characteristics marking sex differentiation. The sex of the silkworm is not to be tampered with by gorging or starving, and what is true of this lepidopteron is undoubtedly true of its cousins, the other moths and the butterflies. It is probably also true of other insects with complete metamorphosis. I recall dissections of various larvæ, notably of *Corydalis cornuta* (a neuropteron) and of *Holorusia rubiginosa* (a dipteron) in which the reproductive organs appear of two sizes in specimens of the same age: indeed in *Corydalis*, of two shapes. These organs need histologic examination. Some student should laboriously work through a long and representative series of insects and settle the question as to the time of sex differentiation. That is, find out whether it be true for all, as it is in the silkworm, that the time of sex differentiation is obvious before, or, at latest, at very little after the time of hatching. If it is true, the question of the influence of nutrition in sex determination will also be settled — for insects. And we need waste no more time in tedious feeding and tabulating.

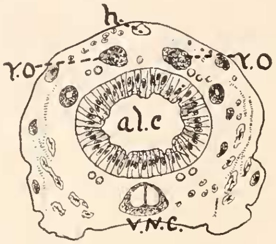

FIG. 8. Section through *Bombyx* larva (male or female ?), two days after hatching; *h*, heart; *al. c*, alimentary canal; *v. n. c*, ventral nerve cord; *r, o*, reproductive organs.

FURTHER NOTES ON THE REGENERATION OF THE FINS OF FUNDULUS HETEROCLITUS.

G. G. SCOTT.

During the last summer the writer while engaged as assistant at the biological laboratory of the U. S. Bureau of Fisheries at Woods Hole, Mass., conducted various experiments on *Fundulus heteroclitus.* Since a number of these can be conveniently grouped under the heading *regeneration* they are collected and presented in the present paper. Various factors determining regeneration have been under discussion and it was thought that these should be tested using *Fundulus h.* as a type since it is a representative teleost and that therefore results might differ from those obtained in crustaceans, for example, and that certain principles deduced from crustacean work might possibly have to be modified. Thanks are due to Dr. F. B. Sumner as director of the laboratory of the Bureau of Fisheries for assistance in providing the necessary apparatus and other facilities.

The following experiments were designed to show: (*a*) the relation of temperature to regeneration, (*b*) the relation of degree of injury to regeneration, (*c*) the relation of length and weight (*i. e.*, age) of specimen to regeneration, (*d*) the relation of amount of food to regeneration, (*e*) adaptation in regeneration.

(A) Relation of Temperature to Regeneration.

With a view of testing this the caudal fin of ten *Fundulus h.* were cut in a line as near as possible to the origin of the caudal fin rays. The same procedure was followed in all the later cases to be described. These fish were placed in a chest or jar with three liters of sea water and the jar placed in an ice chest where the temperature averaged 14°C. The water was changed every day or so. On August 24 five fish had survived. The fish had not been fed. The survivors had been under observations for 41 days or almost six weeks. But in those survivors there was no sign of regeneration. With fishes in normal conditions regeneration is evident after the first few days after operation. In order to further test this point and ascertain whether lack of food had anything to do with the result, two lots of fishes were operated on in a similar manner on September 1. Each lot was placed in a jar in three liters of sea water and both jars placed in the ice chest. One lot was fed and the other not fed. On September 14 nine tenths of those that had not been fed had survived and seven tenths of the others. There was no sign of regeneration in either lot. It might be added that in case of another experiment set up at the same time that regeneration was evident a few days after. (See Table III.)

It may be of interest to note that two of the survivors mentioned in the first experiment were placed in a shallow jar surrounded by running sea water, and in two weeks time the caudal fin showed a regeneration of 2 mm. indicating that just as soon as the temperature was raised to the normal regeneration began. It should also be noted that the temperature necessary to inhibit regeneration was not an extremely low one, and that this indicates that the power of regeneration is sensitive to lowering of temperature.

(B) Relation of Amount of Injury to Regeneration.

In 1902 Zeleny observed that when two chelæ of *Gelasimus*, the fiddler crab, were removed each of the regenerating buds grows more rapidly than does the single one when only one chela is removed. In 1903 the same author found that the regeneration in the arms of the brittle star-fish, *Ophioglypha lacertosa*, varies with the number of arms removed, *i. e.*, that animals with the greater number of arms removed regenerate more rapidly. The greater the degree of injury the more rapid the regeneration. In 1905 Zeleny in an experiment extending over 181 days using the common cray-fish, *Cambarus propinquus*, found that in the series with the greater degree of injury (Series B) each chela regenerates more rapidly than the single chela of those with the lesser degree of injury (Series A). in Series A consisting of 36 individuals the right chela was removed at its breaking joint. In series B consisting of 41 individuals the two chelæ and the last two pairs of walking legs were similarly removed. It is to be regretted that the same author did not include a third series in which the injury was midway between that in A and B to see whether regeneration in that third series would be intermediate between that of series A and B. Thus Zeleny shows that in the cray-fish the greater degree of injury has a stimulating effect on regeneration.

On the other hand Emmel ('04) removed the right cheliped in the lobster immediately after moulting and allowed it to regenerate for the period between moults then removed it again after that moult and allowed it to regenerate. He found that it regenerated less between moults the more it was mutilated. In other words Emmel finds in a general way in a nearly allied form just the opposite result to Zeleny. In 1906 Emmel found a decrease in rate of moulting as correlated with greater degree of injury and lesser rate of regeneration, again a contradictory result to that of Zeleny. With a view of testing this question the following experiment was devised. Three lots of fishes were taken designated as *A*, *B*, *C*.

In *A* the caudal fin was amputated.

In *B* the caudal and one pectoral.

In *C* the caudal and both pectorals.

The fishes were kept in separate compartments in the hatchery with running sea water but were not fed. The experiment was begun on July 30 and continued until September 4 when all were removed and measured with fine pointed registering calipers. In the following Table I. are found the results of these measurements. Not only is the length of the specimen given but the amount of regeneration and the specific regeneration. This is a term used by Zeleny. Since the specimens differ in length we must devise some means of comparing regeneration in one case with that in another. We can do this by finding the percentage regeneration in each case, *i. e.*, by dividing the amount of regeneration by the length of the specimen. We may use Zeleny's term and call it specific regeneration. In the table is also given the weight of each specimen for purposes to be described later.

Now in interpreting these results we have recourse to the statistical method. We should apply certain formulæ here to ascertain whether differences in resulting regeneration in the three different lots are great enough to ascribe any importance to them and thus say that they are due to difference in degree of injury—or on the other hand whether after all they are not simply chance discrepancies. Workers in statistical method have deduced the law that if the mean difference of one character in any two series of forms is less than the probable errors of the difference then the

TABLE I.

Lot A.

	Length Sp.	Amt Reg. Caudal.	Sp. Reg.	Wt. Sp.
1	8.1 cm.	.55 cm.	.0676	6.27 gm.
2	8.0	.65	.0813	5.80
3	6.5	.60	.0923	3.05
4	6.35	.80	.1259	3.38
5	7.25	.65	.0896	4.20
6	9.2	.65	.0706	11.55
7	10.2	.75	.0736	16.70
8	8.25	.80	.0969	8.4
9	9.4	.70	.0744	7.5
10	7.9	.60	.0760	6.25
11	6.35	.60	.0944	3.9
12	8.4	.80	.0952	10.12
13	8.4	.60	.0714	8.85

Lot B.

	Length Sp.	Amt. Reg. Caudal.	Sp. Reg. Caudal.	Amt. Reg. Pec.	Sp. Reg. Pec.	Wt. Sp.
1	11.3	.70	.0610	.55	.0486	26.87
2	7.1	.65	.0915	.50	.0704	4.75
3	8.3	.70	.0843	.60	.0723	7.60
4	8.2	.85	.1037	.60	.0732	10.52
5	7.7	.70	.0909	.60	.0779	5.42
6	11.1	.70	.0631	.65	.0585	22.47
7	8.8	.75	.0852	.60	.0682	9.80
8	7.6	.75	.0987	.60	.0789	11.75
9	9.15	.45	.0491	.45	.0492	10.32
10	6.4	.60	.0937	.50	.0781	2.85
11	5.4	.60	.1111	.50	.0926	2.11
12	6.9	.65	.0942	.60	.0869	4.60
13	5.5	.60	.1090	.30	.0545	2.05
14	8.6	.50	.0581	.50	.0581	5.95
15	6.15	.60	.0975	.50	.0813	3.15

Lot C.

	Length Sp.	Reg Ca.	Sp Reg. Caudal.	Reg. r. Pec	Reg. l. Pec.	Ave. R. L Sp. Reg. Pec	Wt. Sp.
1	11.5	.55	.0478	.55	.55	.0478	23.58
2	9.15	.85	.0929	.45	.45	.0492	12.37
3	9.7	.65	.0670	.50	.45	.0489	11.1
4	7.5	.60	.0800	.60	.60	.0800	5.72
5	7.5	.50	.0666	.35	.40	.0500	4.2
6	10.0	.75	.0750	.70	.70	.0700	14.6
7	6.3	.60	.0952	.50	.55	.0833	4.1
8	6.1	.76	.1065	.40	.50	.0737	3.08
9	5.8	.80	.1379	.45	.40	.0733	2.78

two series do not differ as to that character. If, however, the mean difference is 1 + times the probable difference then it is possible that the two series do differ as to the value of that character. Also if the mean difference is 2 + times the probable difference then it is probable that the two series differ. Finally if the mean difference is 3 + times the probable difference then it is certain that the two series differ as to the value of that character. To apply the law here it is necessary to find the mean of each series, the probable error, and from these compute the mean difference and the probable error of difference.

We can arrange the results as to the caudal fin in Table I. in the form of a table.

Mean Sp. Reg.	Probable Error.	Mean Difference.	Probable Difference.
Lot A = .0860	.003106	Between A and B .0009	Between A and B .004363
Lot B = .0851	.003065	Between B and C .0005	Between B and C .005865
Lot C = .0846	.004991	Between A and C .0014	Between A and C .005876

Comparing A and B we see at once that the mean difference is actually less than the probable difference, and hence this precludes the possibility of any rational conclusion that there is any difference in regeneration in cases A and B. But we have seen that injury in case of A was less than in B. Hence we cannot conclude that the rate of regeneration is greater in the case of the less injured nor in the case of the more injured. The regeneration is the *same*.

We find the same result when we compare B and C, and also when we compare A and C. This experiment tends therefore to negative the results of both Zeleny and Emmel.

We have thus far tested the question by comparing results in these three lots as to regeneration of the caudal fin. But we can also apply the test as to regeneration of the pectorals. We can average the results of the two pectorals in Lot C.

Mean.	Prob. Error.	Mean Diff.	Prob. Diff.
Series B = .0694	.002353		
Series C = .0660	.002752	.0034	.0036

In this case also the mean difference is less than the probable difference hence the two series are the same as to the amount of regeneration in the pectoral fin — although the amount of injury was different in the two cases.

Table II.

Lot D.

	Length Sp.	Amt. Reg. Caudal.	Sp. Reg. Caudal.	Wt. Sp.
1	9.9 cm.	.85 cm.	.0858	16.32 gm.
2	9.2	.70	.0760	13.15
3	8.6	.70	.0818	11.35
4	7.2	.80	.1111	11.52
5	8.9	.70	.0786	6.00
6	7.2	.80	.1111	4.09
7	6.4	.65	.1015	7.10
8	7.1	.85	.1197	6.80
9	7.6	.80	.1052	4.33
10	7.9	.75	.0949	7.22
11	6.1	.70	.1147	5.85
12	7.0	.75	.1071	8.30
13	4.5	.60	.1333	1.37

Lot E.

	Length.	Amt. Reg. Caudal.	Sp. Reg. Caudal.	Amt. Reg. R. Pec.	Sp. Reg. Pec.	Wt. Sp.
1	8.6	.70	.0818	.60	.0697	11.00
2	10.35	.55	.0531	.60	.0579	16.52
3	7.5	.60	.0800	.40	.0500	6.00
4	7.8	.70	.0897	.60	.0897	8.25
5	8.5	.70	.0823	.50	.0588	11.35
6	7.8	.70	.0897	.55	.0705	8.6
7	8.1	.50	.0617	.50	.0617	9.00
8	10.3	.70	.0679	.70	.0679	17.87
9	8.8	.70	.0795	.50	.0568	10.06
10	6.2	.60	.0967	.40	.0646	4.6
11	8.7	.70	.0804	.50	.0574	13.52
12	6.4	.80	.1250	.50	.0781	5.32
13	8.4	.70	.0833	.60	.0714	10.72
14	6.0	.75	.1250	.50	.0833	3.95
15	7.4	.60	.0811	.50	.0675	8.20
16	6.2	.70	.1129	.55	.0887	4.15

Lot F.

	Length.	Amt. Reg. Caudal.	Sp. Reg. Caudal.	Amt. Reg. L. Pec.	Sp. Reg. Pec.	Wt. Sp.
1	7.25	.60	.0827	.60	.0827	5.67
2	7.30	.60	.0821	.35	.0410	8.03
3	7.00	.70	.1000	.50	.0714	6.47
4	6.00	.70	.1166	.60	.1000	4.07
5	6.20	.70	.1129	.40	.0646	4.63
6	6.60	.70	.1060	.60	.1060	5.28
7	6.20	.60	.0967	.50	.0806	3.98
8	6.50	.65	.1000	.40	.0631	5.32
9	6.85	.70	.1022	.60	.0876	3.91
10	6.50	.60	.0923	.45	.0692	5.13
11	6.10	.70	.1147	.50	.0819	4.27
12	6.40	.75	.1172	.35	.1171	4.63

A second experiment was started on August 2 and continued until September 5 a period of four weeks and five days practically the same as those in Table I. In this case the fish were fed regularly for it was desired to test the effect of difference in food on regeneration. In Series D the caudal alone was cut, in E the caudal and right pectoral, in F the caudal and left pectoral. Following is the table of results, Table II.

We may arrange the results as before and have the following table.

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Lot D	.1010	.002956	Between D and E .0155	.004355
Lot E	.0855	.003199	Between D and F .0018	.003977
Lot F	.1028	.002699		

Since but one pectoral fin was cut off in both E and F it is clear that we cannot compare difference in regeneration in those two lots since the degree of injury was the same. In this case we compare E with D and F with D. Here is the first exception to the uniform results obtained in series A, B and C from Table I. For on comparing E with D we find that the mean difference in regeneration is more than three times the probable difference, hence according to our formula regeneration is really greater in D than in E. At first we might think that since the two differ as to degree of injury that this must be the cause of the result — that is, that in this case at least the least injured regenerated more in the same time. But is this the case? For comparing D and F (the injury to F being the same as that to E) we see that the mean difference between the two is less than the probable difference which shows that no importance can be assigned to that mean difference and that D and F regenerated practically the same amount in the same time. The case of D and F confirms the result already found in A–B, B–C and A–C as to caudal, and in case of B–C as to pectoral. The case of D–F also shows that whatever is the cause of the discrepancy between that and D–E we cannot say that E regenerated less because it was injured more than D.

Table III.

Lot G.

	Length Sp.	Amt. Ca. Reg.	Sp. Reg. Ca.
1	6.1	.30	.0491
2	6.2	.25	.0403
3	7.5	.15	.0200
4	6.9	.25	.0362
5	5.9	.30	.0508
6	6.5	.30	.0461
7	7.4	.20	.0270
8	8.1	.30	.0370
9	6.5	.20	.0307
10	7.0	.20	.0285
11	7.0	.30	.0428
12	7.0	.30	.0428
13	6.8	.20	.0293

Lot H.

	Length Sp.	Amt. Ca. Reg.	Sp. Reg. Ca.	Amt. Pec. Reg.	Sp. Reg. Pec.
1	9.0	.20	.0222	.20	.0222
2	7.2	.40	.0555	.20	.0277
3	6.5	.25	.0384	.25	.0384
4	7.7	.20	.0259	.20	.0259
5	6.2	.30	.0484	.25	.0403
6	6.4	.20	.0312	.20	.0312
7	6.8	.30	.0441	.30	.0441
8	7.1	.30	.0422	.25	.0352
9	7.2	.20	.0277	.10	.0138
10	6.4	.30	.0468	.20	.0312

Lot I.

	Length Sp.	Amt. Ca. Reg.	Sp. Reg. Ca.	Amt. Pec. Reg.	Sp. Reg. Pec.	Amt. Reg. Dors.
1	7.3	.30	.0410	.20	.0274	.10
2	6.6	.30	.0454	.25	.0378	.10
3	7.2	.30	.0416	.15	.0208	.10
4	6.5	.25	.0384	.30	.0461	.15
5	7.2	.20	.0277	.20	.0277	.15
6	7.5	.20	.0266	.15	.0200	.10
7	6.4	.30	.0468	.25	.0390	.10
8	7.1	.30	.0422	.25	.0352	.15

In order to test the same question further a third experiment was set up as follows: Three lots of fishes G, H and I were operated on as follows. In G the caudal fin alone was cut off; in H the caudal and one pectoral; in I the caudal, one pectoral and the dorsal. All the fish were placed in a large aquarium with running sea water and were fed regularly. The experiment was begun on September 1 and continued until September 14, 1906. Since all the fish were kept in the same tank there is opportunity for ascertaining whether the discrepancy in case of lot E might not be due to some condition peculiar to the compartment in which they were kept during the five weeks they were under observation. If the results in case of G–H and I confirm the results in cases A–B, A–C, B–C, D–F, etc., then I think we are warranted in concluding that some other condition other than degree of injury was responsible for that discrepancy in case of E. The fishes in this third experiment were fed every day or so. Following is Table III. giving the measurements of these fishes.

Treating these results as before we have the following table :

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Lot G	.0370	.001483	Between G and H .0005	.002611
Lot H	.0375	.002149	Between H and I .0013	.003539
Lot I	.0388	.002813	Between G and I .0018	.003179

We thus see that in this case also the mean difference is not great enough to ascribe any importance to it. In each case it is less than the probable difference and hence although the series differ as to degree of injury, they do not differ as to rate of regeneration. We can also tabulate the comparative regeneration in the case of the pectorals I and H.

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Lot H	0.0312	.001871		
Lot I	0.0320	.001985	.0008	.002727

In this case also the mean difference is less than three times the probable difference which but corroborates the above contention. We therefore find that in nine cases the result is that although the fishes differed as to degree of injury, the rate of regeneration was the same. The experiments are certainly varied enough to represent different lengths of time, etc. There is but one exception to the results and that we have shown cannot be due to difference in degree of injury.

(C) Relation of Length and Weight (i. e., Age) of Specimen to Amount Rate of Regeneration.

Zeleny ('03) observed that the rate of regeneration in the arms of the brittle star-fish, *Ophioglypha lacertosa*, varies with the size of the animal — that the medium sized animals have the maximum rate of regeneration. We quote from his summary, "There is a definite relation between the size (age) of the animal and the rate of regeneration. The maximum rate is exhibited by individuals of medium size. Both the larger and the smaller ones give a diminishing rate as we go away from this point." Zeleny measures size and thus age by the disk width. The three tables given in this paper furnish us with data for determining whether regeneration in *Fundulus* is greater in the smaller, medium, or larger individuals. It occurred to the writer that we ought not only to take length but weight as an indication of age. Unfortunately the specimens in Table III. were not weighed and hence will not be made use of in this comparison. It has been shown by others that regeneration is a phenomenon closely related to growth. Minot has established the fact that growth is greatest in the younger forms. Hence we should expect *a priori* that regeneration should also have a greater rate in the young than in the older forms. For the purposes of this study we can take the regene-

Table IV.

Shortest.			Medium.			Longest.		
Length.	Amt. Reg.	Sp. Reg.	Length.	Amt. Reg.	Sp. Reg.	Length.	Amt. Reg.	Sp. Reg.
6.5	.60	.0923	8.1	.55	.0676	10.2	.75	.0735
6.35	.80	.1259	8	.65	.0813	11.3	.7	.0610
7.25	.65	.0896	9.2	.65	.0706	11.1	.7	.0631
6.35	.60	.0944	8.25	.8	.0969	11.5	.55	.0478
7	.65	.0915	9.4	.7	.0744	10.0	.75	.0750
6.4	.60	.0937	8.4	.8	.0952			
5.4	.60	.1111	8.4	.6	.0714			
6.9	.65	.0942	8.3	.7	.0843			
5.5	.60	.1090	8.2	.85	.1037			
6.15	.60	.0975	7.7	.7	.0909			
6.30	.60	.0952	8.8	.75	.0852			
6.10	.65	.1065	7.6	.75	.0987			
5.8	.80	.1375	9.15	.45	.0491			
7.5	.60	.0800	8.6	.5	.0581			
7.5	.50	.0666	9.15	.85	.0929			
			9.7	.65	.0670			
			7.9	6.0	.0760			

ration in the caudal fin of all the fishes represented in Table I. This irrespective of the fact that they differ as to degree of injury, since it has just been shown that that has not affected the rate of regeneration. In Table I, we have 37 specimens — the shortest is 5.4 cm. long while the longest is 11.5 cm. long. We can divide the 37 fishes into three lots (1) the shorter, those between 5.4 cm. and 7.5 cm.; the medium between 7.6 cm. and 9.7 cm. while the longer are between 9.8 cm. and 11.9 cm. Arranging all the specimens in these three groups we have the results shown in Table IV.

We find that the average specific regeneration of the shorter is .1028, that of the medium is .0797, while that of the longer is .0638. In other words, the shortest have regenerated 10 + per cent. of their own length, the medium-sized have regenerated 7 + per cent. of their length, while the longest have regenerated 6 + per cent. of their length. Thus the result is contrary to that found by Zeleny with the brittle star-fish. But to make certain we can employ the statistical method used above and have the following table.

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Shortest	.0972	.002823	Between S and M =.0190	.003698
Medium	.0782	.002390	Between M and L =.0096	.003786
Longest	.0686	.002934	Between S and L =.0286	.004071

Now we find that the mean difference between S and M is not only more than three times the probable difference but nearly six times as large so that there can be no question but that the shortest have regenerated at a greater rate. On comparing S with L we find that the mean difference in regeneration is over seven times the probable difference. So that our conclusion is that the shorter have regenerated at a much greater rate than either the medium or the longer and that also the medium have regenerated at a greater rate than the longest. Now this last is confirmed when we compare M and L. In this case the mean difference is not quite three times the probable difference but more than twice the probable difference so that it is more than probable that the medium have regenerated more than the longer.

But we also have Table II. for comparison. The shorter specimens in this series are those between 4.5 cm. and 6.45 cm; the medium between 6.46 and 8.41 and the longer between 8.42 and 10.37 cm. Arranging the specimens we have Table V.

Table V.

Shorter.			Medium.			Longer.		
Length.	Amt. Reg.	Sp. Reg.	Length.	Reg.	Sp. Reg.	Length.	Reg.	Sp. Reg.
6.4	.65	.1015	7.2	.8	.1111	9.9	.85	.0858
6.1	.7	.1147	7.2	.8	.1111	9.2	.7	.0760
4.5	.6	.1333	7.1	.85	.1197	8.6	.7	.0818
6.2	.6	.0967	7.6	.8	.1052	8.9	.7	.0786
6.4	.88	.1250	7.9	.75	.0949	8.6	.7	.0818
6	.75	.1250	7	.75	.1071	10.35	.55	.0531
6.2	.7	.1129	7.5	.6	.0800	8.5	.7	.0823
6	.7	.1166	7.8	.7	.0897	10.3	.7	.0679
6.2	.7	.1129	7.8	.7	.0897	8.8	.7	.0795
6.2	.6	.0967	8.1	.5	.0617	8.7	.7	.0804
6.1	.7	.1147	8.4	.7	.0843			
6.4	.75	.1172	7.4	.6	.0811			
			7.25	.6	.0827			
			7.3	.6	.0821			
			7	.7	.1000			
			6.6	.7	.1060			
			6.5	.65	.1000			
			6.8	.7	.1022			
			6.5	.6	.0923			

Treating the results as before, we find the average specific regeneration for the shortest is .1136, that of the medium is .0944 while that of the longest is .0763. In other words the shortest of this series of fed fishes have regenerated 11 + per cent. of their length, the medium sized have regenerated 9 + per cent. of their length while the longest have regenerated 7 + per cent. of their length.

In this case also we can apply the statistical method tabulating our results in the following form.

	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Shortest	=.1137	.002897	Between S and M =.0203	.003492
Medium	=.0934	.001949	Between M and L =.0170	.003066
Longest	=.0764	.002367	Between S and L =.0373	.003741

Here again it will be seen that the mean difference in regeneration between S and M is over three times the probable difference, in fact nearly six times; the mean difference between S and L shows that it is over nine times the probable difference so that there can be no doubt that the shortest specimens in Table II. regenerated at a more rapid rate than did the medium or the longest. On comparing the medium with the longest we find that the medium regenerated more rapidly than the longest as is evidenced by the fact that the mean difference is over five times the probable difference.

If we extend our hypothesis that there is a direct relation between growth and regeneration further it would be to find out whether as the fish grew older, the rate of regeneration decreased. For this is precisely the condition as regards growth — the rate of growth decreases with age. At first the decrease is rapid but then becomes very gradual. This is indicated with regard to regeneration. For on comparing regeneration of the shortest with the medium and the regeneration in the medium with the longest we discover an indication of this relation. In each of the two cases it is seen that mean difference in regeneration is greater between shortest and medium as compared with the mean difference in regeneration between the medium and longest.

It was said at the beginning of this section that in estimating age we ought to consider both length and weight. So I have placed opposite the length, etc., of each specimen its weight. *A priori*, the youngest ought to be the shortest and lightest, etc. This is the case here, for the average weight of the shortest is 3.38 gm.; the average weight of the medium length is 8.96 gm., while that of the longest is 20.85 gm. So that in Table IV. our three classes represent the youngest, the oldest and those midway between the others in age. In the same way for Table V. the average weight of the shortest is 4.48 gm.; that of the longest is 12.71 gm. while that of the medium length is 7.52 gm. In this case also our three series in Table V. represent the youngest, the oldest and the medium aged fishes of that lot. We conclude therefore that the rate of regeneration in *Fundulus h.* is greatest in the youngest fishes, less in the older, and that there is a slight indication that the rate of regeneration decreases with age.

(D) Relation of Regeneration to Food.

It will be remembered that fishes in Series A, B and C were not fed while those in Series D, E and F were fed. Otherwise as far as known they were kept under identical conditions and for the same length of time. It was desired to compare regeneration in the two series with each other to ascertain whether regeneration is at a greater rate in the fed fishes than in the not-fed. Unfortunately we cannot make use of Series E because as before said some factor has entered there and caused a result which I do not believe to be normal. On the other hand I do regard Series D and F as normal. Therefore we must compare regeneration in D with that in A.

I. Caudal.	Mean Reg.	Prob. Error.	Mean Diff	Prob. Diff.
Lot D (fed)	.1010	.003106		
Lot A (not fed)	.0860	.002958	.0150	.004249

In this case the mean difference in regeneration is more than three times the probable difference. In other words the fishes in Lot D which were fed have regenerated more in the same time than the fishes in Lot A which were not fed.

II. Caudal.	Mean Reg.	Prob. Error.	Mean Diff.	Prob. Diff.
Lot F (fed)	.1028	.002659		
Lot B (not fed)	.0851	.003065	.0177	.004084

In this case also the mean difference in regeneration between F and B is more than three times the probable difference. Or the fishes in Lot F which were fed have regenerated more than the fishes in Lot B which were not fed.

We may compare the average specific regeneration of the caudal in the case of the fishes that were fed with that in the fishes that were not fed in the following way also.

Average Sp. Reg. in caudal of Lots D + F (fed).............................. = 0.10100
Average Sp. Reg. in caudal of Lots A + B + C (not fed).................... = 0.08565
Difference.. = 0.01535

The caudal fin in fishes that were fed regenerated 18 per cent. more than that in fishes not fed.

Again,

Average Sp. Reg. in pectoral fin in Lot F (fed).................................. = 0.0866
Average Sp. Reg. in pectoral fin in Lot B + C (not fed)...................... = 0.0677
Difference.. 0.0189

that is, the pectoral in the fed fishes regenerated 24 per cent. more than that in the unfed.

(E) Regeneration in Fins used most Compared with that in Less used Fins.

Adaptation in Regeneration. — Osburn ('06) experimenting on the fins in *Fundulus* finds that the pectorals are not used as vigorously as the caudal, and the dorsal not so much as the pectorals. Broussonet (1786) stated that the most useful fins regenerate more rapidly than those less useful. Morrill ('06) says that the "caudal fin though obviously the most important does not regenerate perceptibly faster than the others." Although the data are insufficient to settle this question which is to a great extent a matter of interpretation yet the following table is suggestive. Since it has been shown that degree of injury has made no difference in the rate of regeneration in *Fundulus h.*, it is therefore possible to group all the specimens represented in Table I. thus,

Average Sp. Reg. of caudal fin in Lots A + B + C........................... = 0.08565
Average Sp. Reg. of pectoral fin in Lots B + C...... .... = 0.06770
Difference.. = 0.01795

which means that the caudal fin of specimens in Lots A + B + C regenerated 26 per cent. more than the pectoral fin in Lots B + C in the same time and under same conditions.

In the same way from Table II., we find

Average Sp. Reg. of caudal fin in Lots D + F........ .... = 0.01016
Average Sp. Reg. of pectoral fin in Lot F.. = 0.00846
Difference .... = 0.00170

or in other words the caudal regenerated 20 per cent. more than the pectoral.

Again in Table III. the

Average Sp. Reg. in caudal in Lots G + H + I................................ = 0.0386
Average Sp. Reg. in pectoral in Lots H + I...................................... = 0.0313
Difference .... = 0.0073

or the caudal in this case has regenerated 20 per cent. more than the pectoral. Thus in all three cases the result is the same. It should be remembered that specific regeneration is the relation of amount regenerated to the length of the specimen. And hence we have a right to compare the specific regenerations of caudal with pectoral. It may also be pointed out here that the dorsal fin regenerates very slowly as compared with the other two as may be seen in Table III.

GENERAL CONCLUSIONS.

1. That low temperatures inhibit regeneration in caudal fin of *Fundulus heteroclitus*.

2. That the rate of regeneration bears no relation to the degree of injury to the caudal and pectoral fins.

3. That regeneration is greater in the younger than in the medium and older fishes which is in line with the theory that regeneration is a growth phenomenon.

4. That regeneration is greater in the fish that have been fed as compared with that in the fishes not fed.

5. That there *is* an indication that the fins used or needed most (*i. e.*, caudal) regenerate more rapidly than the less needed fin (pectoral), or in other words there is an indication of adaptation in regeneration.

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